LONGITUDINAL IMAGING AND CLINICAL ASSESSMENT OF PATIENTS WITH UNRESECTABLE HEPATOCELLULAR CARCINOMA TREATED WITH TRANSCATHETER ARTERIAL CHEMOEMBOLIZATION

by

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Abstract

Statement of problem: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death and often arises in the context of hepatic cirrhosis. Transcatheter arterial chemoembolization (TACE) is a non-surgical treatment for patients with unresectable HCC involving local and targeted intra-arterial delivery of chemotherapy. Currently, it is unclear whether overall survival in patients with HCC treated with TACE can be predicted by longitudinally measured clinical and imaging biomarkers. The aim of this study was to determine whether longitudinally collected metrics might contribute to the prediction of overall patient survival.

Materials and Methods: This IRB-approved cohort study included 211 consecutive patients with unresectable HCC treated with TACE between 1/1/2001 and 11/28/2008. A complete case analysis was performed on baseline and longitudinally collected clinical and imaging data of 119 patients. Baseline (time-independent) and longitudinal (time-dependent) data included age, gender, ethnicity, type of cirrhosis and Eastern European Cooperative Group (ECOG) performance status. Time-dependent data included Child-Turcotte-Pugh (CTP) score, tumor burden, longest tumor size, portal vein thrombosis, tumor response according to Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1 and modified RECIST. Overall survival estimates were calculated by the Kaplan-Meier method. Cox regression models were used to predict the effect of time-independent and time-dependent variables on overall survival.

Results: Mean overall survival was 18.85 months (median 14, range: 1-148). The analysis of time-independent Cox models revealed that portal vein thrombosis (HR=

2.47, 95% CI= 1.32-4.64, p=0.01) and uni-dimensional tumor size greater than 5 cm (5-7.99 cm, HR= 3.16, 95% CI=1.27-7.90, p=0.01; 8-11.99 cm, HR=3.37, 95% CI=1.37-8.30, p=0.01; >12 cm, HR=3.56, 95% CI= 2.46-22.53, p<0.0001) were independent baseline predictors of decreased overall survival, after adjusting for ethnicity and etiology of cirrhosis. The analysis of time-dependent Cox models revealed that an ECOG status of 1 or 2 (adjusted HR=1.83, 95% CI=1.11-3.03, p=0.02,) and a CTP score of B (adjusted HR=1.50, 95% CI=1.07-2.12, p=0.02), were independent time-varying predictors of decreased overall survival.

Conclusion: The results of our statistical analyses using time-dependent variables highlights the prognostic value of longitudinally collected markers and should alert clinicians to the value of their collection and assessment.

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CHAPTER 1. INTRODUCTION

In clinical oncologic research studies, repeatedly or longitudinally measured disease imaging and biological markers are used to monitor treatment and disease progression. Analysis of longitudinal data, such as clinical and/or radiologic metrics of response to therapy, biochemical and/or genetic biomarkers, or health-related quality of life outcomes, may aid in predicting a time to event, such as relapse-free survival or overall survival. Longitudinal studies are becoming increasingly used, since they may provide a better and growing understanding of the risk of death in patients who are repeatedly evaluated over time.

The goal of this work is to present and investigate methods for assessing the association between repeatedly measured clinical and imaging markers of disease control and overall survival in patients with unresectable hepatocellular carcinoma (HCC) who are treated with transcatheter arterial chemoembolization (TACE). Our study hypothesis is that longitudinally measured tumor imaging and clinical data in such patients are superior predictors of overall survival than imaging and clinical data collected at baseline. To test our hypothesis, we will examine prospectively collected imaging and clinical data from patients with unresectable HCC treated with TACE. TACE is an established treatment for patients with unresectable HCC and is commonly administered more than once in each patient. The physician decision to retreat is based on a combination of endogenous parameters, such as the degree of liver function deterioration together with the degree of tumor response to therapy, for each patient. Although TACE

is a locally targeted therapy, some non-targeted chemotherapy deposition in the surrounding hepatic parenchyma occurs and further deteriorates an already compromised liver function. Because HCC very often appears in patients already having a cirrhotic liver background, it is important to describe patients' changes in liver function and tumor response to therapy over time.

The primary purpose of this work is to determine whether baseline and longitudinal estimates of tumor response to therapy and liver function scores are predictive of overall survival in patients with unresectable HCC treated with TACE. The outline of this work is as follows. Chapter 2 presents background information describing the epidemiology and diagnosis of HCC, the TACE procedure and the current evidence of effectiveness and efficacy in patients with unresectable HCC, the imaging criteria for evaluating tumor response to therapy and the classification system for evaluating liver cirrhosis. Chapter 3 presents a description of the methods used for evaluating longitudinal and survival data. Chapter 4 presents the results of the statistical analyses obtained using the Johns Hopkins Hospital HCC TACE database. Finally, in Chapters 5 and 6, respectively, conclusions are presented and future directions are discussed.

CHAPTER 2. BACKGROUND

2.1. Epidemiology And Diagnosis Of Hepatocellular Carcinoma

Each year, hepatocellular carcinoma (HCC) is diagnosed in more than 500,000 people worldwide, with approximately 20,000 new cases in the United States, where HCC is the fastest growing cause of cancer-related death (Hashem B. El-Serag, 2011; H. B. El-Serag & Davila, 2011). HCC incidence rates are increasing in the United States and many other parts of the world, not only due to improved surveillance methods, but also possibly due to the obesity epidemic and the rise in HCV infection (Davis, Alter, El-Serag, Poynard, & Jennings, 2010; Hashem B. El-Serag, 2011; Jemal et al., 2011). Other notable risk factors for the development of HCC, such as HBV infection and lack of immunization against HBV or alcoholic cirrhosis may also contribute to the increase of HCC incidence rates. Notably, unlike most other solid cancers, prevalence and mortality rates for HCC are projected to increase over the next 20 years (Davis et al., 2010).

The diagnosis of HCC is based on the results of typical radiological features in at least two imaging modalities including ultrasound, magnetic resonance imaging (MRI), contrast-enhanced dynamic computed tomography (CT), and hepatic arterial angiography, or by a single positive imaging technique accompanied with serum α-fetoprotein (AFP) level >400 ng/mL or by histology(Bruix et al., 2001). Despite the fact that improved methods of surveillance utilization have increased the number of patients with HCC at earlier stages of disease progression, most patients with HCC already have

advanced and unresectable disease at the time of diagnosis and are being thus offered only non-surgical treatment options (H. B. El-Serag & Davila, 2011; Liapi & Geschwind, 2007, 2010a, 2010b; B. H. Zhang, Yang, & Tang, 2004). Additionally, almost 80% of patients with unresectable HCC have underlying liver cirrhosis(H. B. El-Serag & Rudolph, 2007; D. Poon et al., 2009). This results in a degree of complexity that is not present in some other cancer types, making optimal treatment choice a challenge.

2.2. Transcatheter Arterial Chemoembolization For Treatment Of Unresectable Hepatocellular Carcinoma

Among non-palliative treatment options for patients with unresectable HCC, intra-arterial therapies offer targeted anti-cancer therapy with local intra-arterial hepatic injection, using x-ray image-guidance (Liapi & Geschwind, 2007). The most commonly performed intra-arterial therapies include transcatheter arterial chemoembolization (TACE) with ethiodized oil, transcatheter arterial chemoembolization with drug-eluting beads (DEB-TACE), hepatic arterial embolization (HAE) and radioembolization (RE) (Lewandowski, Geschwind, Liapi, & Salem, 2011; Liapi & Geschwind, 2007, 2010a, 2010b, 2011). TACE is currently the most widely performed therapy throughout the world and is the focus of the current work.

Transcatheter arterial chemoembolization (TACE) with ethiodized oil (or lipiodol) for treatment of unresectable HCC was originally introduced in the 1980s (Yamada et al., 1980; Yamada et al., 1983). TACE involves the local intra-arterial hepatic injection of one or more chemotherapeutic agents along with ethiodized oil and embolic materials.

The chemotherapy (commonly doxorubicin or cisplatin) is suspended in ethiodized oil (an oily contrast medium selectively retained within tumors) and injected via a catheter into the hepatic arteries that directly supply the tumor. The tumor feeding arteries are subsequently infused with embolic materials, most commonly microspheres, so as to decrease blood flow to the tumor and allow prolonged retention of chemotherapy to the tumor (Liapi & Geschwind, 2007, 2010a, 2010b, 2011; Liapi, Lee, Georgiades, Hong, & Geschwind, 2007).

Almost fifteen years ago, two randomized controlled trials (RCT) demonstrated a significant survival benefit in patients with unresectable HCC undergoing TACE versus best supportive care (Llovet et al., 2002; Lo et al., 2002). Since then, TACE has been considered as the standard of care in patients with unresectable HCC(Basile, Carrafiello, Ierardi, Tsetis, & Brountzos, 2012). In these two RCT studies, however, TACE was compared against best supportive care in selected patients with well-preserved liver function (Llovet et al., 2002; Lo et al., 2002). Moreover, different chemotherapeutic agents and embolic materials were used in each of these studies. Since then, several observational cohort studies and case series have shown the efficacy of TACE (Liapi & Geschwind, 2007, 2010a, 2010b, 2011; Takayasu et al., 2006). Meta-analyses and systematic reviews, however, have demonstrated conflicting findings regarding the benefit of TACE, thus far (Forner, Llovet, & Bruix, 2012; R. S. Oliveri, Wetterslev, & Gluud, 2011; Roberto S. Oliveri, Wetterslev, & Gluud, 2012). Under the umbrella of the TACE procedure, investigators may often include procedures that do not require injection of chemotherapy, such as the procedure of lipiodolization or the procedure of hepatic

arterial embolization (HAE). It has been argued, however, that tumor ischemia alone, induced by these procedures may not lead to tumor death (Rose et al., 2013). Whereas an initial meta-analysis demonstrated a survival benefit for patients treated with TACE over best supportive care, a more recent one could not support evidence that either TACE or hepatic arterial embolization (HAE) has a beneficial effect on survival in patients with unresectable HCC (Forner et al., 2012; Llovet & Bruix, 2003; R. S. Oliveri et al., 2011; Roberto S. Oliveri et al., 2012). This could be attributed to the introduction of new variations of the TACE procedure, such as the DEB-TACE, or variations in the chemotherapy and embolization protocols.

2.3. Child-Turcotte-Pugh Classification Method of Liver Cirrhosis Severity

Liver cirrhosis is the final common histologic pathway for a wide variety of chronic liver diseases, such as alcoholic liver disease, or hepatitis C (Heidelbaugh & Bruderly, 2006). Liver cirrhosis is a severe condition for which overall survival is the principal endpoint (Durand & Valla, 2005). In addition, HCC occurs in the setting of liver cirrhosis in up to 80% of patients (H. B. El-Serag & Davila, 2011; H. B. El-Serag & Rudolph, 2007; D. Poon et al., 2009).

The most commonly used method to classify patients according to the extent of liver cirrhosis is the Child-Turcotte -Pugh (CTP) classification (Pugh, Murray-Lyon, Dawson, Pietroni, & Williams, 1973). This classification provides a simple method for

identifying patients at risk for liver insufficiency. Child and Turcotte initially developed this classification in 1964 and Pugh modified this in 1973 (Child & Turcotte, 1964; Durand & Valla, 2005; Pugh et al., 1973). The current CTP scoring system is based upon five parameters: serum bilirubin, serum albumin, prothrombin time, ascites and encephalopathy. The total score, corresponding to the sum of individual points across the five parameters (**Table 1)**, allows categorizing patients by CTP grades A (5-6 points), B (7-9 points) and C (10-15 points). Of note, the total range of points (5-15) is not equally distributed among grades A, B and C, signifying the additive clinical impact of the each grade in terms of prognosis. These grades have been shown to correlate with the following one and two- year survival rates: Grade A, 100% and 65%; Grade B, 80% and 60%; and Grade C, 45% and 35%, respectively (Durand & Valla, 2005, 2008). A recent systematic review found that the median survival was ≤6 months in patients with cirrhosis CTP score ≥12 (Salpeter, Luo, Malter, & Stuart, 2012).

Table 1. Child-Turcotte-Pugh (CTP) classification of liver cirrhosis severity. The total score, corresponding to the sum of individual points across the five parameters, allows categorizing patients by CTP grades A (5-6 points), B (7-9 points) and C (10-15 points).

Parameter	**Points Assigned**		
	1	**2**	**3**
Ascites	Absent	Slight	Moderate
Bilirubin (mg/dL)	<2	2 to 3	>3
Albumin (g/dL)	> 3.5	2.8 to 3.5	< 2.8
Prothrombin Time Seconds prolonged	1 to 3	4 to 6	>6
INR	< 1.7	1.8 to 2.3	> 2.3
Encephalopathy	None	Minimal	Advanced

INR: International Normalized Ratio. Prothrombin time values of 4 and 6 seconds correspond approximately to 50% and 40% of normal, respectively. Either Prothrombin Time or INR is used for calculating the final score.

The original score was initially proposed in the limited setting of the evaluation of surgery for portal hypertension (Child & Turcotte, 1964). Subsequently, its prognostic value was demonstrated in the settings of ascites (Merkel et al., 2000), variceal bleeding (Fernandez-Esparrach et al., 2001), subclinical encephalopathy (Hartmann et al., 2000), small untreated HCC (Barbara et al., 1992), abdominal surgery (Mansour, Watson, Shayani, & Pickleman, 1997), alcoholic cirrhosis (Gluud, Henriksen, & Nielsen, 1988), decompensated HCV-related cirrhosis (Planas et al., 2004), primary sclerosing cholangitis (Shetty, Rybicki, & Carey, 1997), primary biliary cirrhosis (van Dam et al., 1999) and Budd-Chiari syndrome (Zeitoun et al., 1999).

The CTP score has not been rigorously validated in patients treated with intra-arterial therapies. However, decompensated liver cirrhosis (CTP class C with numeric score > 12) has long been considered to be a contraindication to the intra-arterial approach, not only because of the possibility of severe complications following the procedure but also due to the poor prognosis despite treatment in such patients (Liapi, Georgiades, Hong, & Geschwind, 2007). Surprisingly, the effect of intra-arterial therapies on the cirrhotic liver has not been reported very extensively (Adhoute, Penaranda, Castellani, Perrier, & Bourliere, 2015; Ogasawara et al., 2015). In the case of TACE in patients with cirrhosis, few authors have reported on the effect of the procedure on liver function and the damage caused by TACE to non-tumorous liver tissue (Khan et al., 1991).

2.4. Radiologic Evaluation of Hepatocellular Carcinoma Response To TACE

Imaging quantification methods for evaluating the treatment response of liver tumors to transcatheter arterial therapies, including TACE, provide treating physicians with an insight of the effect of chemotherapy and embolization on disease progression. Current size-based criteria for assessing cancer response to therapy include the World Health Organization (WHO) criteria and the Response Evaluation Criteria in Solid Tumors (RECIST) (Eisenhauer et al., 2009; Miller, Hoogstraten, Staquet, & Winkler, 1981).

The WHO criteria were the first criteria established for the recording and reporting of solid tumor response to therapy, in 1979 (Miller et al., 1981). These criteria attempted to standardize the recording and reporting of response assessment for solid tumors, so as to allow comparisons between research studies throughout the world. Assessment of tumor response according to the WHO criteria is performed by measuring the product of the longest axial diameter and greatest perpendicular diameters for each tumor. Complete response (CR) is defined as the disappearance of all signs of disease for at least 1 month and the lack of appearance of new lesions. Partial response (PR) is achieved with a 50% or more reduction in the sum of the tumor cross product sizes. Stable disease (SD) indicates a decrease of <50% or an increase in tumor size of <25% over the original

measurements. Progressive disease (PD) is considered a 25% or more increase in the sum of one or more of the tumor measurements or the appearance of new lesions.

In 1994, several organizations, including the European Organization for Research and Treatment of Cancer, the National Cancer Institute of USA and the National Cancer Institute of Canada began to review the response assessment criteria with the intent of revising them. The revised system, the Response Evaluation Criteria In Solid Tumors (RECIST), used the longest only axial tumor diameter (uni-dimensional) for each tumor (and up to 5 tumors per target organ), which are less cumbersome to measure and calculate (Therasse et al., 2000). The revised RECIST version 1.1 guidelines were established in 2009 and use uni-dimensional tumor measurements of up to two target lesions per organ (Eisenhauer et al., 2009). A sum of the longest diameter for these two target lesions is calculated and reported as the baseline sum of the longest diameters. To characterize the objective tumor response, the baseline sum of the longest diameters is used as the reference. Complete response (CR) is disappearance of all known lesion(s) confirmed at 4 weeks. Partial response (PR) is 30% or more decrease in the sum of the longest diameters confirmed at 4 weeks. Stable disease (SD) is when neither PR nor PD criteria are met. Progressive disease (PD) is 20% or more increase in the sum of the longest diameters and an absolute increase of 5 mm for each target lesion or appearance of new lesion(s). Lymph nodes are considered a measurable and possible target lesion if they measure more than 15 mm in the short axis.

The relevance of the application of the RECIST criteria to TACE has been challenged, since TACE may lead to tumor ischemia and necrosis that may not always be

paraleled by a reduction in size (Bruix et al., 2001; Lencioni & Llovet, 2010). More recently the American Association for the Study of Liver Diseases (AASLD) has proposed a formal amendment of the RECIST criteria to take into consideration changes in the degree of tumor arterial enhancement – the modified RECIST criteria (mRECIST) (Lencioni & Llovet, 2010). According to the mRECIST, the following RECIST modifications were proposed in the determination of tumor response for target lesions: CR is considered the disappearance of any intratumoral arterial enhancement in all target lesions. PR is considered at least a 30% decrease in the sum of longest diameters of viable (contrast enhancement in the arterial phase) target lesions, taking as reference the baseine sum of the longest diameters of target lesions. PD is characterized as an increase of at least 20% in the sum of the diameters of viable (enhancing) target lesions, taking as reference the smallest sum of the longest diameters of viable (enhancing) target lesions recorded since the treatment started. SD includes any cases that do not qualify for either PR or PD (**Table 2**).

Table 2. Radiologic Imaging criteria for evaluating tumor response to therapy: comparison of the RECIST v.1.1 and mRECIST.

	RECIST	**mRECIST***
CR	Disappearance of all target lesions	Disappearance of any intratumoral arterial enhancement in all target lesions
PR	At least a 30% decrease in the sum of longest diameters of target lesions, having as reference the baseline sum of the longest diameters of target lesions	At least a 30% decrease in the sum of longest diameters of viable (enhancement in the arterial phase) target lesions, having as reference the baseline sum of the longest diameters of target lesions
SD	Any cases that do not qualify for either partial response or progressive disease	Any cases that do not qualify for either partial response or progressive disease
PD	An increase of at least 20% in the sum of the longest diam-ters of target lesions, having as reference the smallest sum of the longest diameters of target lesions recorded since treatment started	An increase of at least 20% in the sum of the diameters of viable (enhancing) target lesions, having as reference the smallest sum of the longest diameters of viable (enhancing) target lesions recorded since treatment started

*Adapted from Lencioni R, Llovet JM(Lencioni & Llovet, 2010). Abbreviations: mRECIST, modified Response Evaluation Criteria in Solid Tumors; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Currently, there is little evidence whether longitudinally measured RECIST and mRECIST scores are superior in predicting HCC patients' overall survival, compared to metrics obtained at baseline. Our hypothesis is that longitudinally measured tumor imaging and clinical biomarkers of liver function are superior predictors of OS than biomarkers measured at baseline.

2.5. Patient Performance Status

Several patient performance status instruments are available to clinicians for evaluating patient performance status. In oncology, the most commonly used are the Karnofsky Performance Scale and the Eastern Cooperative Oncology Group (ECOG) scale.

The Karnofsky Performance Scale was developed in the 1980s as an attempt to measure the more subjective aspects of the outcome of cancer treatment (Mor, Laliberte, Morris, & Wiemann, 1984). The scale only relates to the physical ability. It quickly and easily indicates how the patient is feeling on a given day. The Karnofsky Performance Scale covers 11 points, from normal health to death. Each is scored from 0 to 100.

The performance status scale developed by the Eastern Cooperative Oncology Group (ECOG) was developed in the1980s as an attempt to measure the more subjective aspects of the outcome of cancer treatment(Oken et al., 1982).The ECOG scale records activity on a scale from 0 (fully active, able to carry on all pre-disease performance without restriction) to 5 (dead). **Table 3** provides a description of scoring values of the ECOG performance status scale. Physicians routinely use these criteria and scales to evaluate the progression of cancer and how the daily living ability of patients is affected. In addition, performance status is used as an indicator of treatment and predictor of long-term survival. However, few studies have specifically investigated the influence or prognostic ability of performance status on HCC patients (Hsu et al., 2013; Nishikawa et al., 2015).

Table 3. Description of scoring values of the ECOG performance status scale (Oken et al., 1982).

Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self care, confined to bed or chair for more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

2.6 Specific Aims

Because the relationship between overall survival (OS) and longitudinal measures of radiologic tumor response and liver function deterioration after TACE have not been extensively studied in patients with unresectable HCC, we sought to determine whether any of these repeatedly obtained measurements can predict OS in a single center prospective study. Our study hypothesis is that longitudinally measured tumor imaging and clinical variables of clinical performance and liver function are superior predictors of OS than variables measured at baseline. To test our hypothesis, we examined prospectively collected tumor imaging and clinical liver function data from patients with unresectable HCC treated with TACE and sought to:

Specific Aim 1: Evaluate whether baseline patient characteristics could predict overall survival.

Specific Aim 2: Evaluate whether longitudinally obtained measurements with time-varying covariates could predict patient overall survival.

CHAPTER 3. METHODS

3.1 Study Design

This study is a HIPAA-compliant, IRB-approved single center observational study of consecutive patients with unresectable HCC treated at the Johns Hopkins Hospital (JHH) with TACE, between January 2001 and November 2008. Data were retrieved from JHH electronic patient records.

Inclusion criteria were: diagnosis of hepatocellular carcinoma (HCC) according to imaging and/or laboratory criteria, treatment with TACE between 1/1/2001 and 11/28/2008, at least one imaging study of the liver, at least one laboratory biochemical evaluation of the liver with clinical evaluation of ascites and encephalopathy. Exclusion criteria were: treatment with any other intra-arterial therapy other than TACE (such as drug-eluting beads TACE, radioembolization, or hepatic arterial embolization), before, during and at the end of the study period. Patients receiving transplantation or had recurrent disease were excluded from the study. Patients treated with previous TACE sessions outside the JHH were excluded from the study. Patients who had previously received systemic chemotherapy, surgery, ethanol or radiofrequency ablation, were also excluded from the analysis. For the longitudinal data analysis, patients included in the study had to have baseline and last follow-up (exit) imaging and liver function tests.

Therefore, patients without baseline and/or exit information on their CTP score, RECIST and mRECIST scores were subsequently excluded from the study.

Administrative censoring occurred at the study end date (11/28/2008). A total of 211 patients were initially eligible. A total of 92 patients were excluded, as they did not fulfill the repeated measures study entry criteria. Thus, 119 patients were included in the repeated measures analysis. The patients were observed until either the primary endpoint (death) or administrative censoring (11/28/2008) occurred. Vital status information was obtained on patients via a private Social Security Death Index Registry. **Figure 1** shows a flowchart of patients included and excluded from the repeated measures study.

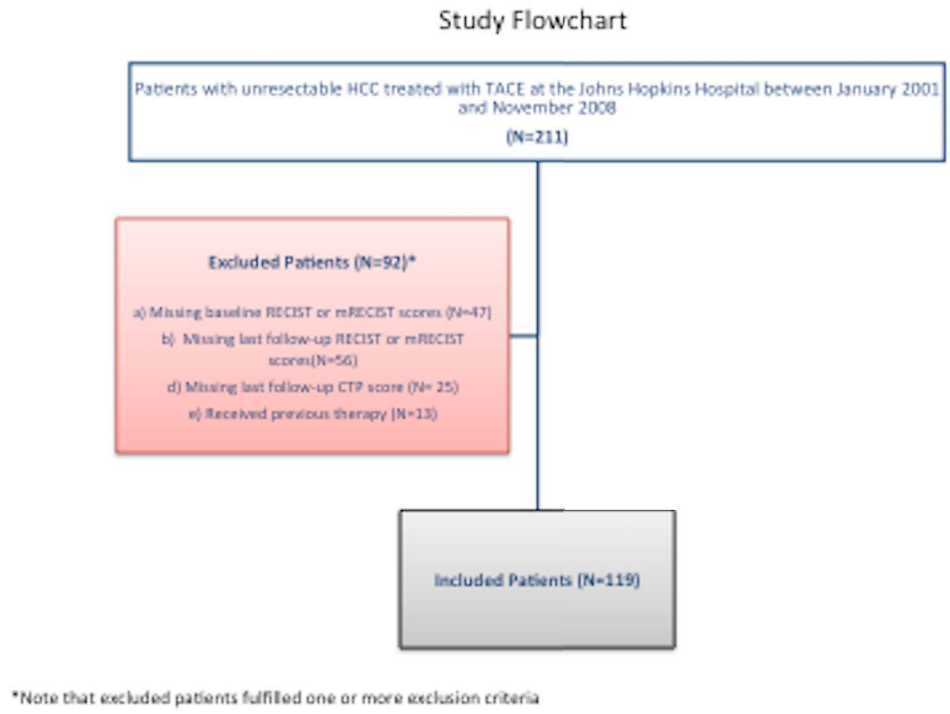

Figure 1. Flow chart of patients with unresectable HCC treated with TACE at the JHH between 2001 and 2008. Patients could be excluded for multiple reasons.

3.2 Johns Hopkins Hospital TACE Treatment and Follow-up Protocol

The Johns Hopkins Hospital TACE treatment protocol consists of selective or supraselective TACE with use of 50 mg doxorubicin, 100 mg cisplatin and 10 mg of mytomicin C mixed with ethiodized oil, followed by the injection of embolic microspheres. The procedural steps can be summarized as follows:

The single-wall Seldinger technique with an 18-gauge needle is used to access the right common femoral artery. A 5-French vascular sheath is then placed into the artery over a 0.035 glide wire. Under fluoroscopic guidance, a 5-French catheter is then used to select the superior mesenteric artery (SMA) and the celiac axis. The SMA angiography is essential to identify variant and hepatic accessory arteries, retrograde flow though the gastroduodenal artery (GDA), and visualize portal vein patency. Over the guide wire, the 5-French catheter is next advanced into the desired hepatic artery branch, depending on tumor location. TACE is then performed with supraselective catheterization of the hepatic arteries that feed a tumor and intra-arterial injection of a solution containing cisplatin 100 mg, doxorubicin 50 mg and mitomycin C 10 mg in a 1:1-2:1 mixture with ethiodized oil. This is followed by injection of 3-6 ml of a mixture containing embolic microspheres (100–300 μm in size) suspended in 1:1 ratio of contrast medium. The technical endpoint of TACE is successful delivery of chemotherapy with targeted ethiodized oil deposition, reduction of arterial inflow to the tumor and tumor de-vascularization (Liapi, Georgiades, et al., 2007).

The JHH TACE evaluation and follow-up protocol includes: a) a baseline clinical visit, during which baseline imaging, clinical evaluation and liver function tests were obtained, b) TACE treatment, c) first follow-up visit, 4-6 weeks after first TACE, during which imaging, clinical evaluation and liver function tests were obtained. A subsequent TACE session was performed, only if all of the following conditions were met: a) presence of persistent untreated disease, indicated by imaging, b) adequate liver function, indicated by liver function tests that did not show CTP score C, c) absence of post-

operative complications, such as liver abscess, d) patient's agreement to continue therapy. Patients with no remaining untreated disease were closely observed with imaging and liver function tests every 4- 6 weeks and until recurrent untreated disease occurred. The 2nd follow-up visit occurred, either 4-6 weeks after the second TACE, or 2-3 months after the first TACE. Similarly, the 3rd follow-up visit occurred, either 4-6 weeks after the third TACE session, or 4-6 months after the first TACE session. Subsequent sessions occurred at unpredictable intervals, based on the endogenous algorithm assessment of each biochemical and imaging follow-up, which occurred every 4-6 weeks, irrespectively of therapy. For simplicity purposes, we consider each visit as a landmark time point and the last follow-up visit was the last before study exit.

3.3 Imaging Protocol

All radiological assessment was performed using magnetic resonance imaging (MRI). The MRI protocol included gradient echo T1-weighted (T1 GRE) fat suppressed sequences before and after intravenous injection of gadolinium (Gd) agent, turbo spin-echo T2-weighted (T2 TSE) sequences (Kamel et al., 2009; Kamel, Reyes, Liapi, Bluemke, & Geschwind, 2007).

All treated lesions were measured on the arterial phase of post-Gd T1 GRE dynamic sequences according to: a) RECIST criteria, by measuring the percentage of change in the sum of the maximal bi-dimensional perpendicular diameters and the maximal uni-dimensional diameter, including viable and non-enhancing areas within the tumor, and b) mRECIST criteria, by measuring the percentage of change in the sum of

the maximal bi-dimensional diameters and the maximal uni-dimensional diameter, including only the enhancing portion of the tumor. For these response criteria, radiologic interpretation was classified as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) according to cutoffs defined in Chapter 2. Measurements were repeated at each follow-up visit until study exit.

3.4 Study Variables

In this study, we prospectively collected time-independent (baseline) and time-dependent (repeatedly measured) variables. **Table 4** shows the baseline variables: age at study entry, gender, ethnicity, etiology of hepatic cirrhosis, presence of extrahepatic metastases and baseline clinical performance status.

Table 4. Time-independent baseline covariates, measured at baseline only, of patients with unresectable HCC treated with TACE at the JHH.

Variable Name	**Variable Type**	**Variable Description**	**Coding Index**
Age	Continuous	Age in years, calculated at the time of baseline clinical visit to the Division of Interventional Radiology	
Sex	Binary	Gender	Male=0 Female=1
Ethnicity	Categorical	Ethnicity	White=0 African American=1 Asian/Pacific Islander=2 Other=3
Hepatic Disease	Categorical	Etiology of cirrhosis	Unknown/cryptogenic=0, Hepatitis-B related cirrhosis=1, Hepatitis-C related cirrhosis=2, Alcoholic cirrhosis=3, Combination of HBV and HCV-related cirrhosis=4, Combination of alcoholic and HBV=5, Combination of alcoholic and HCV=6, Combination of alcoholic and HBV/HCV=7 Hemochromatosis=8, Hemochromatosis and HBV=9, Hemochromatosis and HCV=10, Non-alcoholic steatohepatitis (NASH)=11, None=12
ECOG	Ordinal	Clinical performance status	Fully Active=0 Light Work=1

Table 5		according to the European Cooperative Oncology Group (ECOG) guidelines at baseline	No work=2
Extrahepatic Metastases	Categorical	Presence of extrahepatic metastasis	None=0 Regional Lymph Nodes=1 Distant Metastases=2

Repeatedly measured variables, were measured longitudinally, at baseline, 1st, 2nd and last recorded visits, and included imaging and biochemical variables, as listed in **Table 5.** Imaging variables included the longest axial tumor size, tumor response by RECIST and mRECIST, presence of portal vein thrombosis, presence of untreated tumor(s), and tumor burden. All measurements were taken from computed tomography or magnetic resonance imaging scans interpreted by at least one JHH staff radiologist. Biochemical parameters included the numerical and categorical CTP scores, as well as the individual variables that comprise these scores (albumin, bilirubin, prothrombin time, ascites, and encephalopathy).

Table 6. Repeatedly measured covariates of patients with unresectable HCC treated with TACE at the Johns Hopkins Hospital

Variable Name	Variable Type	Variable Description and Coding Index	
CTP Numerical Score	Continuous	Numerical CTP score	Range: 5-15
CTP Categorical score	Categorical	Categorical CTP score,	A (5-6 points)=1 B (7-9 points)=2 C (10-15 points)=3
Uni-dimensional Tumor Size	Continuous	Longest unidimensional axial tumor diameter, measured in centimeters (cm)	
RECIST	Ordinal	Tumor response to therapy according to the RECIST v1.1 criteria	Complete Response (CR)=0 Partial Response (PR)=1 Stable Disease (SD)=2 Progressive Disease (PD)=3
mRECIST	Ordinal	Tumor response to therapy according to the mRECIST criteria	Complete Response (CR)=0 Partial Response (PR)=1 Stable Disease (SD)=2 Progressive Disease (PD)=3
PVT	Binary	Portal vein thrombosis of the main or any segmental portal vein branch	No=0 Yes=1
Untreated tumors	Binary	Presence of non-targeted tumors, as recorded on imaging studies of the liver, binary	No=0 Yes=1
Tumor Burden	Categorical	Tumor burden, described as the total number of tumors	Single tumor=1 Two or three tumors=2 More than three tumors=3
Extrahepatic Disease	Categorical	Presence of extrahepatic disease	No=0 Regional lymph nodes=1 Distant metastases=2

* CTP scores, presence of PVT, extrahepatic metastases, tumor burden, were measured at baseline, first follow-up, second follow-up and last visit. RECIST, mRECIST scores and presence of untreated disease were recorded at first follow-up, second follow-up and last visit.

3.5 Study Endpoint

The primary outcome of this study was overall survival (OS) and was measured in months from baseline imaging assessment until either death, or at the time point at which there was loss to follow-up or administrative censoring.

3.6 Statistical Methods

Statistical analysis included both exploratory data analyses and survival analysis utilizing time-independent and time-dependent variables.

3.6.1 Exploratory Data Analysis

Exploratory data analysis (EDA) aided in study hypothesis building and included non-graphical and graphical methods of data analysis. Univariate EDA included summary statistics of each continuous variable with respect to central tendency (mean, median), spread (standard deviation, variance, interquartile range), shape (skewness, kurtosis) and outliers. Distributions of continuous variables were explored using dot plots, box plots, and quantile-normal plots. Distributions of categorical variables were explored using frequency distributions.

Bivariate associations were inspected visually through side-by-side box plots, two-way plots and scatterplots, and cross-tabulations. Bivariate analysis were performed using Mann Whitney's U test, the Student *t* test, chi-square test, or Fischer's exact test, as

appropriate, to compare baseline characteristics between excluded and included patients. Comparisons of baseline characteristics between these two groups were performed for the following variables: a) age (with the Wilcoxon rank-sum test), b) gender, c) ethnicity, d) type of hepatic cirrhosis, e) ECOG patient performance status, f) presence of extrahepatic disease, g) tumor size, h) CTP score and i) portal vein thrombosis, using the Pearson's chi-squared test. Comparisons for detecting differences in baseline tumor size measurements among the three baseline RECIST categories and the four baseline mRECIST categories were performed suing Kruskal-Wallis H-test. Scatter graphics representing the percentage of change in longest axial tumor size at each visit were built and spaghetti plots of RECIST, RECIST, and categorical CTP measurements were constructed, considering all follow-up visits until study exit. Box and whisker box plots showing median, range, and interquartile values, were constructed to investigate changes between baseline and the 1st, 2nd and last visits, in RECIST, mRECIST, and CTP scores, visually as well as with Friedman's two-tailed test and Wilcoxon's test. Tumor radiological response and liver function classification were represented by summary statistics and graphical methods. For all tests, a *P* value <0.05 was considered statistically significant. All analyses were conducted using STATA 12 software (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP).

3.6.2 Kaplan-Meier Survival Analysis

Kaplan-Meier estimates of the cumulative probability of survival were used to account for censored and uncensored data. Censored data were defined when the event

time of interest was not fully observed on all subjects under study, for instance if they were lost to follow up or dropped out of the study, or if the study ended before they died.

Since survival data were expected to be right skewed, we used rank-based non-parametric tests followed by estimate and confidence intervals of medians within groups. Kaplan-Meier estimates were computed and compared for all baseline characteristics. Comparisons between groups were performed using the log-rank test(Kaplan & Meier, 1958; Mantel, 1966; Peto & Peto, 1972). When comparisons included more than two groups, a Bonferroni correction was applied by dividing the assumed significance level by the number of comparisons and the log-rank test p-value represented the p-value for the trend.

3.6.3 Multivariable Cox Proportional Hazards Regression Analysis of Baseline Covariates

A univariate Cox regression analysis was first performed to identify baseline predictors of the risk of death(Cox, 1972). The Efron approximation was employed for tied survival times(Efron, 1977). Characteristics examined at baseline included age, gender, ethnicity, type of hepatic cirrhosis, CTP score, ECOG performance status, presence of extrahepatic metastases, uni-dimensional longest tumor diameter (categorical), tumor burden, as well as portal vein thrombosis. RECIST, mRECIST at the first visit were also considered as baseline characteristics and were included in the univariate analysis. Predictors were further considered for inclusion in the multivariate

proportional hazards regression analysis only if they had achieved a p-value of <0.2(Greenland, 1989).

Covariates were selected for inclusion in the multivariable Cox proportional hazards regression model (Cox, 1972) using stepwise or forward selection methods and according to the level of statistical significance as describe above. Caution was taken to avoid multi co-linearity by excluding variables that were highly correlated.

3.6.4 Evaluation of Proportional Hazards Assumption Model for Baseline Covariates

Cox proportional hazard modeling was evaluated for supporting the assumption of proportional hazards graphically and by employing the Schoenfeld test. In order to graphically assess the proportional hazards assumption for each model, we generated plots of the logarithm of time against the logarithm of the negative logarithm of the estimated survivor function for each variable ("log-log" survival curves). For each plot, if curves were not crossing each other, then PH assumption was considered to be satisfied.

For each covariate, the Schoenfeld residuals are defined as the value of individuals that failed minus its expected value. We subsequently performed analysis of Schoenfeld residuals, by fitting a smooth function of time to the residuals (Schoenfeld, 1982). If the residual exhibits a random (i.e. unsystematic) pattern at each failure time, then this suggests that the covariate effect is not changing with respect to time and the PH assumption is met. If it is systematic, it suggests that as time passes, the covariate effect is changing.

The Schoenfeld test (stphtest) was then used to test the independence between residuals and log time(Grambsch & Therneau, 1994). The Schoenfeld test is analogous to testing whether the slope of scaled residuals on time is zero or not. If the slope is not zero then the proportional hazard assumption has been violated. The Schoenfeld test will be performed for each covariate and globally using a formal significance test. If a baseline covariate in a model did not meet the proportional hazards assumptions, we further stratified models by this covariate.

3.6.5 Cox Proportional Hazards Regression Analysis using Time-Varying Covariates

In the classical Cox regression model analysis, the prognostic factors represent a subject's condition at the start of the study (e.g. age at baseline, performance status, tumour characteristics) and do not change over time. In this study, the biomarkers of tumor response and liver function change over time and it is of interest to take this into account when modelling overall survival. Therefore, a Cox regression model with time-dependent covariates was considered (Collet 2003). In this model, the hazard h of death is modelled as a function of time t, for the i-th of n subjects

$$h_i(t) = \exp\left\{\sum_{j=1}^{p} \beta_j x_{j,i}(t)\right\} h_0(t)$$

In this expression $h_0(t)$ is the baseline hazard function and $x_{i,j}(t)$ represents the value of the j-th covariate for the i-th subject at time t. The parameter β_j, $with\ j = 1, \ldots, p$

corresponds to the log hazard ratio for two individuals whose values of x_{ij} at any time *t* differ by one unit, all other variables being the same at that time. Note that in this model, as the values of $x_{ij}(t)$ may vary over time, the ratio $h_i(t)/h_0(t)$ is also time-dependent and the model is no longer a proportional hazards model. The classical Cox regression model however follows as a special case where $x_{ij}(t) = x_{ij}$ for all t. Furthermore note that this model requires the values of the time-dependent covariates to be specified for each individual in the risk set at each event time.

Each study visit was considered as being spaced 2 months apart from a subsequent one because this was the assumed time window for follow-up visits. . Panel data were set up in the following manner: a) patients that survived less than 1 month were excluded from the study, b) patients that survived the 1st visit and died before the end of the 2 months period were censored at 1 month, c) patients that survived the 2nd visit and died before the end of 4 months period were censored at 3 months, d) patients that survived the 3rd visit and died and died before the end of 6 months were censored at 5 months. In order to perform a complete case analysis, missing values of RECIST, mRECIST and CTP scores were dropped from the analysis.

3.6.6 Model Selection

When a number of models are fit to the same data set, one method of choosing the 'best' model is to select the model for which Akaike's information criterion (AIC) is lowest (Akaike, 1974). AIC applies when maximum likelihood is used to estimate the unknown parameters in the model. The value of -2 log likelihood for each model fit is

penalized by adding twice the number of estimated parameters. The number of estimated parameters includes both the linear parameters and parameters in the covariance structure. Another criterion for model selection is the Bayesian information criterion (BIC)(Schwarz, 1978). BIC penalizes -2 log likelihood by adding the number of estimated parameters multiplied by the log of the sample size.

Model selection was performed only for time-independent models. Model selection for time-dependent models is considered as future work.

CHAPTER 4. RESULTS

4.1. Exploratory Data Analysis

4.1.1. Patient Demographic Characteristics

From the total of 211 TACE patients, 119 were included in the longitudinal analysis study group and 92 were excluded. The median age in the study group was 61.5 years (inter-quartile range= 51-69 years, mean=60.45 years, SD=13.34, normally distributed with Shapiro-Wilk test p-value=0.70). Most patients were male (83.20%), Caucasian (66.90%) and with hepatitis C-related cirrhosis (29.40%). Baseline demographic characteristics of patients included in the study, compared to those excluded from the study, are shown in **Table 6.** There were no statistically significant differences between the two groups, with respect to age (p=0.32, Wilcoxon rank-sum test), ethnicity (p=0.18, Pearson's chi-squared test) and etiology of hepatic cirrhosis (p=0.59, Pearson's chi-squared test). There was a statistically significant difference in the gender distribution (p=0.023, Pearson's chi-squared test), with a higher proportion of female patients in the study group, as compared to those excluded from the study. There was also a statistically significant difference in the distribution of patients with extrahepatic metastases (p=0.047, Pearson's chi-squared test), with a higher proportion of patients with enlarged regional lymph nodes included in the study. The total numbers of observations for each measured variable are shown in **Table 7**.

Table 7. Baseline demographic characteristics of patients with unresectable HCC treated with TACE for patients included as compared to patients excluded from the longitudinal study.

Factor	Included	%	Excluded	%	P-value	Test
N	119		92			
Age, median (IQR)	61 (51, 69)		63 (53, 71)		0.32	Wilcoxon rank-sum
Gender					**0.023**	Pearson's chi-squared
Male	99	83.2	87	93.5		
Female	20	16.8	6	6.5		
Ethnicity					0.18	Pearson's chi-squared
White	79	66.9	54	58.1		
African American	22	18.6	23	24.7		
Asian	9	7.6	13	14.0		
Other	8	6.8	3	3.2		
Etiology of hepatic cirrhosis					0.59	Pearson's chi-squared
Unknown/ cryptogenic	34	28.6	32	34.40		
Hepatitis B	23	19.3	11	11.80		
Hepatitis C	35	29.4	31	33.30		
Hepatitis B and alcohol	5	4.2%	4	4.30		
Other*	22	18.5	15	16.10		
Baseline ECOG Status					0.84	Pearson's chi-squared
0	80	67.2	61	65.6		
1 or 2	39	32.8	28	30.1		
Baseline Extrahepatic Disease					**0.047**	Pearson's chi-squared

None	91	76.50	82	88.20%		
Regional lymph nodes	14	11.80	3	3.20		
Distant metastases	14	11.80	8	8.60		
Baseline Tumor size (cm)					0.069	Pearson's chi-squared
2-4.99	24	26	27	41		
5-7.99	24	26	20	30		
8-11.99	32	34	13	20		
12-16	14	15	6	9		
Baseline CTP score					0.25	Pearson's chi-squared
A	80	67.20	56	70.90		
B	36	30.30	18	22.80		
C	3	2.50	5	6.30		
Baseline Portal Vein Thrombosis					0.65	Pearson's chi-squared
No	89	74.80	67	72.00		
Yes	30	25.20	26	28.00		

*Includes combination of alcoholic and HCV cirrhosis, combination of alcoholic and HBV/HCV cirrhosis, hemochromatosis, hemochromatosis and HBV, hemochromatosis and HCV, non-alcoholic steatohepatitis, or none

Table 8. Total number of observations for each measured variable for patients included and excluded from the study. Parentheses indicate the percent missing data for each measured variable.

	Included Observations	Missing Values (%)	Excluded Observations	Missing Values (%)
	N=119		N=92	
Age	118	0.8	92	0
Gender	119	0	92	0
Ethnicity	118	0.8	92	0
Hepatic cirrhosis	119	0	92	0
Baseline ECOG status	119	0	92	0
Metastases	119	0	92	0
Baseline CPT score	119	0	92	0
Baseline tumor size	94	21	66	28.3
Baseline PVT	118	0.8	92	0
1st RECIST score	119	0	47	48.9
1st mRECIST score	119	0	47	48.9
1st CPT score	99	16.8	24	73.9
2nd RECIST	118	0.8	53	42.4
2nd mRECIST	118	0.8	53	42.4
2nd CPT score	100	16	24	73.9
Last RECIST	119	0	56	39.1
Last mRECIST	119	0	56	39.1
Last CTP score	119	0	25	72.8

Missing data for patients included in the study were less than 10% for each measured variable, with the exception of baseline tumor size (21%) and CTP score at 1st follow-up visit (16.8%) and CTP score at 2nd follow-up visit (16%).

The median number of TACE sessions per patient was 2 (mean=2.64, SD=1.63 sessions). Most patients (39.5%) received 2 TACE sessions, where as 15.6% received 3 sessions. **Figure 2** shows the distribution of TACE sessions per patient.

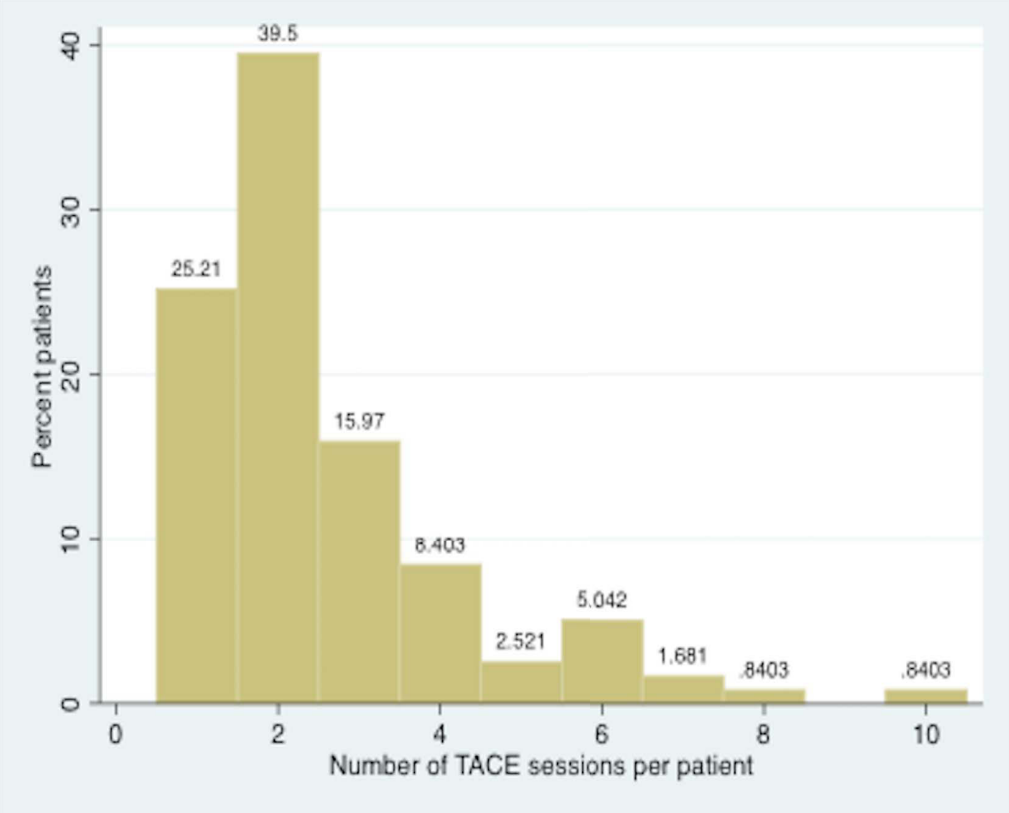

Figure 2. Distribution of the number of TACE sessions per patient.

The baseline mean uni-dimensional longest tumor diameter was 7.76 cm (SD=3.62 cm, median=7.5cm, inter-quartile range=4.7-10 cm; not normally distributed by Shapiro-Wilk test, p-value=0.01). **Figure 3** shows the distribution of baseline uni-dimensional tumor size by baseline CTP score (Kolmogorov-Smirnov test for equality of distribution functions, p=0.92). **Figure 4** shows the distribution of baseline uni-dimensional tumor size by gender. **Figure 5** shows the distribution of baseline uni-dimensional tumor size by 1st RECIST tumor response. No statistically significant

differences for baseline uni-dimensional tumor size measurements were observed among the three baseline RECIST categories (Kruskal-Wallis H-test p-value: 0.80).

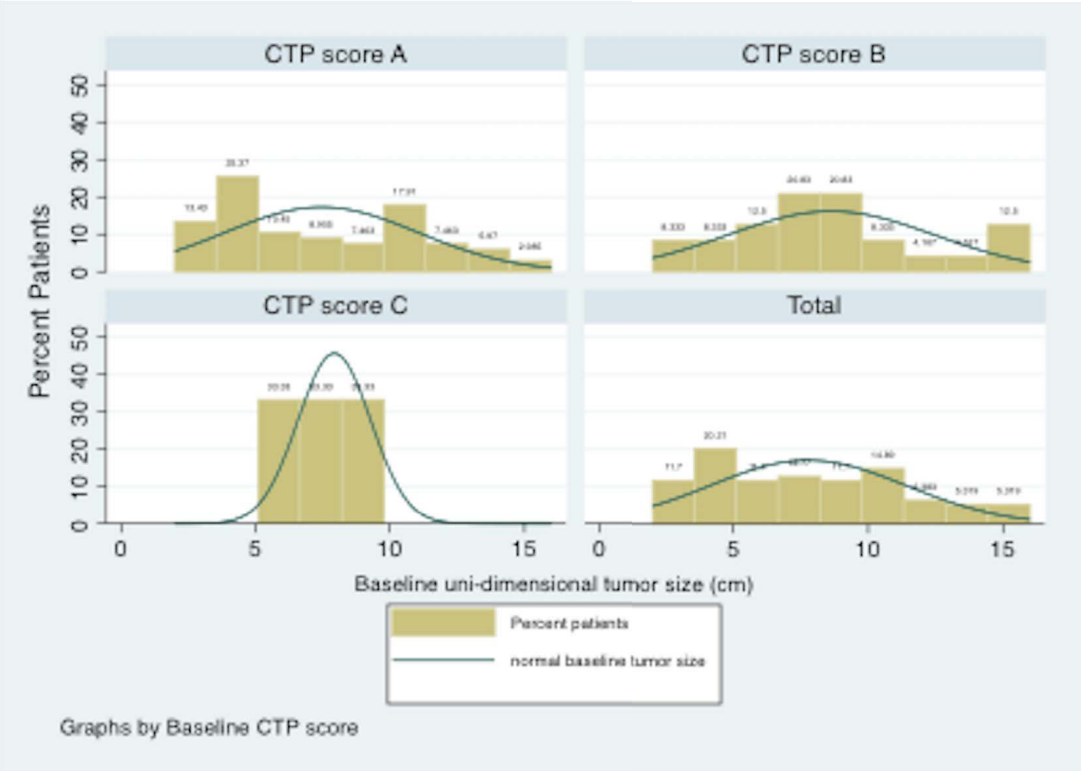

Figure 3. Distribution of baseline uni-dimensional tumor size (in cm) across patients (percent patients) by CTP score.

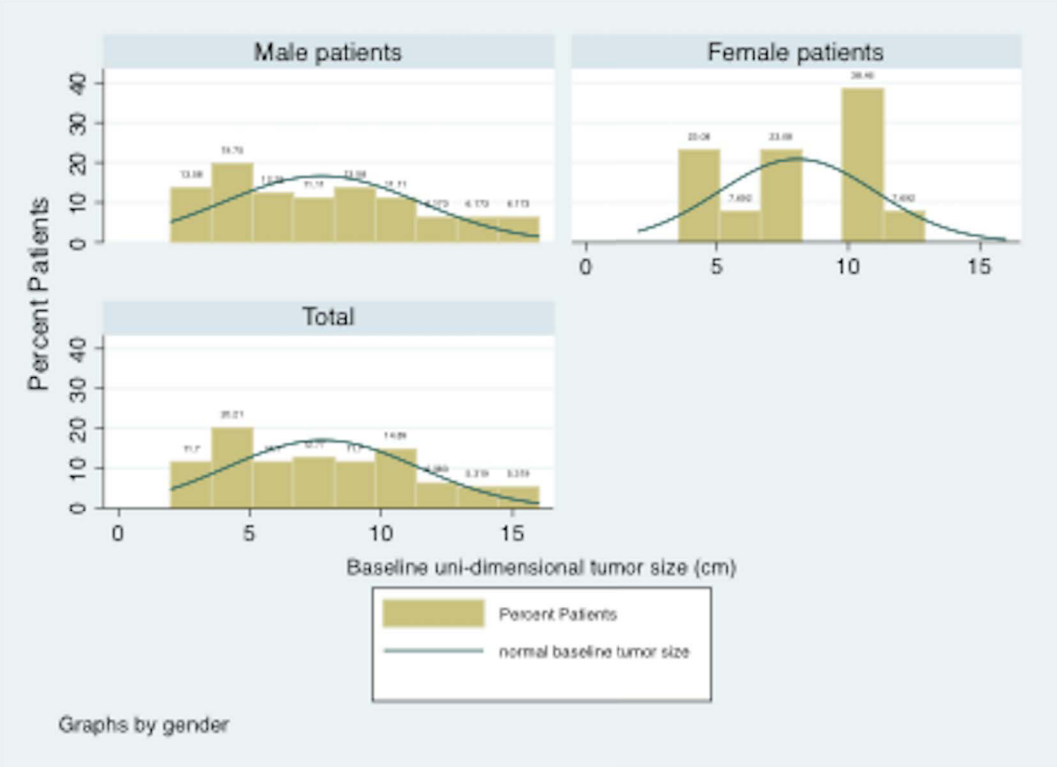

Figure 4. Distribution of baseline uni-dimensional tumor size (in cm) across patients (percent patients) by gender.

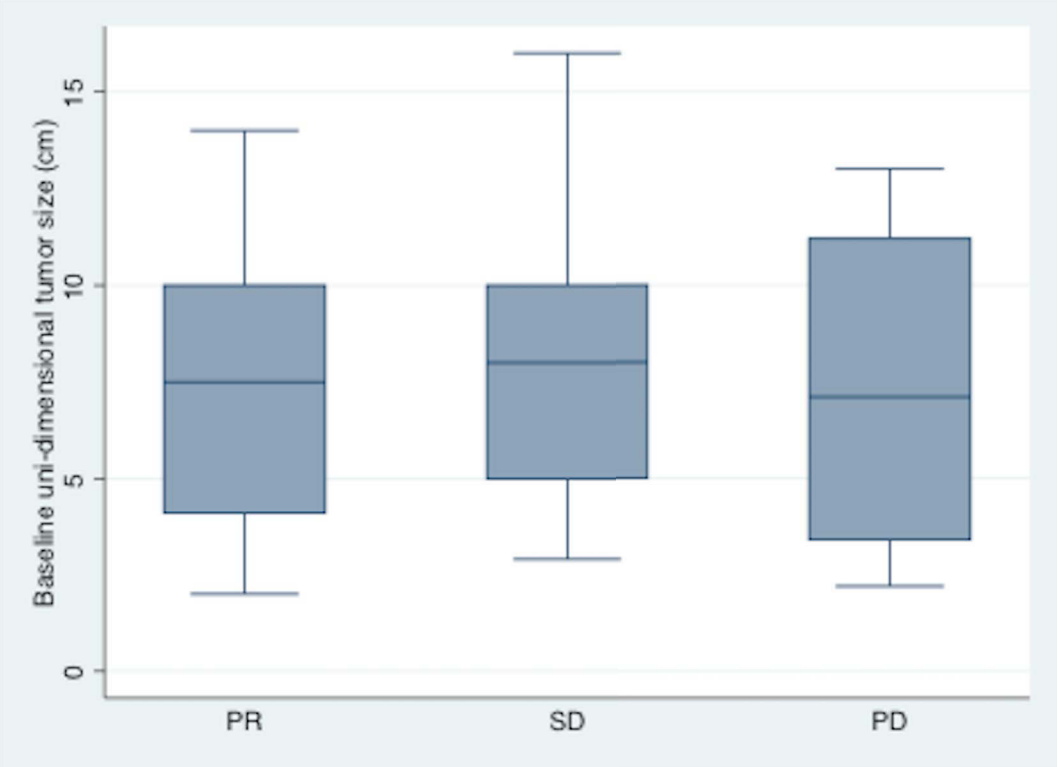

Figure 5. Box and whisker plots of baseline uni-dimensional tumor size (cm) by baseline RECIST categories (p=0.80, Kruskal-Wallis test). PR=partial response, SD= stable disease, PD=progressive disease.

4.2. Analysis of Trends Over Time

4.2.1. Assessment Of Liver Function Over Time

Our study population consisted of 119 patients that had primarily a baseline CTP score of A (n=80), while 36 had CTP score B and 3 had advanced liver cirrhosis (CTP score C). After the first TACE session, patients were re-evaluated in a subsequent visit (1st follow-up visit) with calculation of the numeric and categorical CTP score. Either after the 2nd TACE session or after 4-6 weeks from the 1st visit, patients were asked to follow up with a subsequent visit (2nd visit). The last visit was the last visit prior to study exit and varied for each patient. At the 1st follow-up visit, 60 patients had a CTP A score, 32 had CTP B score and 7 had a CTP score of C, with 20 missing values. At the 2nd follow-up visit, 60 patients had a CTP score of A, 28 had CTP B score and 9 had a CTP score C, with 19 missing values. At the final visit, 58 patients had a CTP score of A, 59 had CTP score of B and 16 had a CTP score of C. **Table 8** demonstrates the number of patients and categorical CTP scores at each visit, as well as the total count of each CTP score and the percent change in the counts of each score compared to baseline. At the final visit, there was a 32.5% decrease in CTP score A, a 44.5% increase in CTP score B and a 333% increase in CTP score C. **Figure 6** shows a spaghetti plot of the CTP predicted values using a linear regression model of observed CTP score on visit for each patient.

According to the numerical CTP score, patients had a baseline mean numerical CTP score of 6.17 (SD=1.52, range: 4-13). On the 1st follow-up visit, the mean numerical

CTP score was 6.43 (SD=1.60, range: 3-11, p=0.233, compared to baseline), on the 2nd follow-up visit the mean score was 6.66 (SD=1.68, range: 5-12, p=0.025 compared to baseline) and on the last follow-up the mean CTP score was 7.10 (SD=1.79, range: 5-12, p<0.00001 compared to baseline CTP score. Although there is a statistically significant difference between the 2nd and baseline CTP scores, as well as the last and baseline CTP scores, mean numerical values for each of these visits fall into the categorical CTP score of B, which has a range between 7-9. **Table 9** shows the mean numerical CTP score for each visit and their relevant t-test p-values, when compared to baseline CTP scores.

Table 9. Categorical CTP score distribution at each visit. N=number of patients (%). Change (%) signifies the percent change from baseline.

CTP score	**N (%)**	**N (%)**	**Change (%)**	**N (%)**	**Change (%)**	**N (%)**	**Change (%)**
	Baseline	**1st follow-up**		**2nd follow-up**		**Last follow-up**	
A	80 (67.23)	60 (50.42)	-25.00	61 (51.26)	-23.75	54 (45.38)	-32.50
B	36 (30.25)	32 (26.89)	-11.11	30 (25.21)	-16.66	52 (43.7)	44.46
C	3 (2.52)	7 (5.88)	133.33	9 (7.56)	200.00	13 (10.92)	333.33

Table 10. Numerical CTP score distribution at each visit. Obs=number of observations, SE=standard error of the mean, SD= standard deviation, LCI and UCI=lower and upper 95% confidence intervals, T-test P-values are recorded for comparison of each visit CTP score to baseline.

Numerical CTP score	**Obs**	**Mean**	**SE**	**SD**	**LCI**	**UCI**	**P-value**
Baseline	119	6.18	0.14	1.52	5.90	6.45	
1st follow-up	100	6.43	0.16	1.61	6.11	6.75	0.233
2nd follow-up	99	6.67	0.17	1.68	6.33	7.00	0.0251
Last follow-up	119	7.11	0.16	1.79	6.78	7.43	0.00001

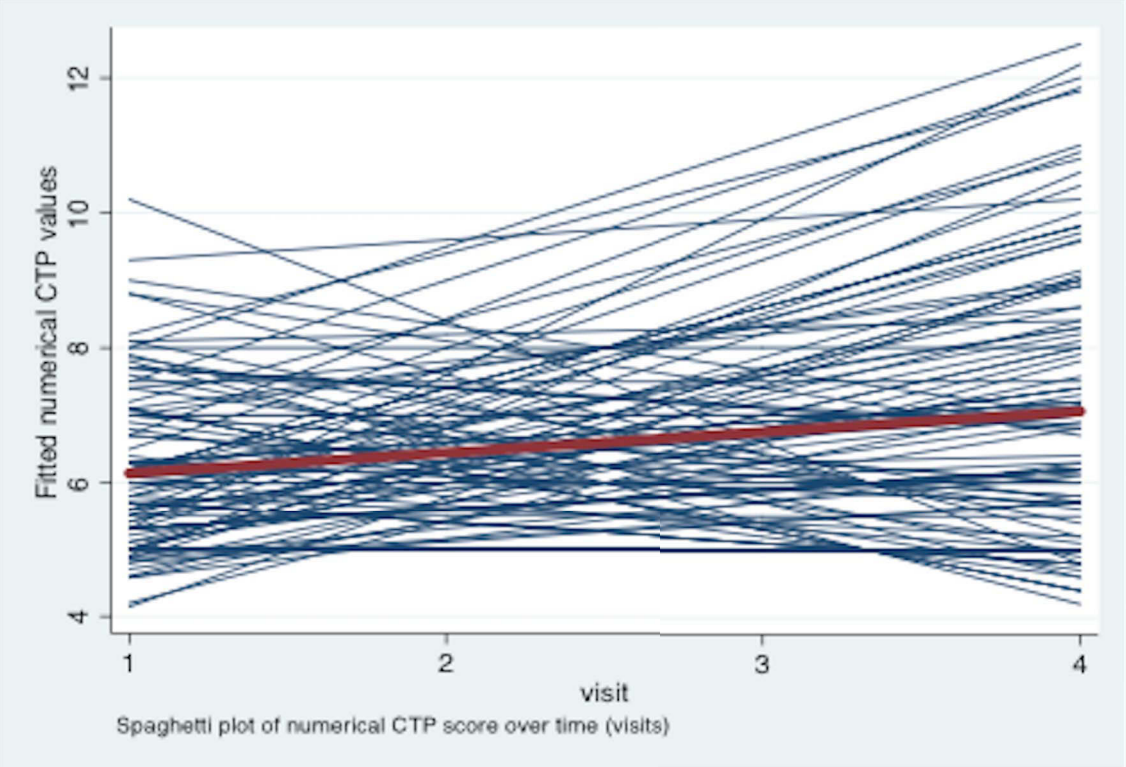

Figure 6. Spaghetti plot of fitted CTP scores by visit for each patient over the study period of three consecutive visits and the last before study exit visit. Visits are visually spaced equally, but not spaced equally in time. The red line represents the overall fitted regression line of CTP on visit number.

4.2.2. Assessment of Tumor Response to Therapy Over Time

For assessing tumor response to therapy, RECIST and mRECIST were employed. Moreover, the longest axial diameter of the largest tumor (labeled as index tumor) was measured and diameter changes were recorded over time. Other descriptive tumor measures included tumor burden (single tumor, 2-3 tumors, or >3 tumors), presence of extrahepatic disease (absence, presence of distant metastases, presence of local lymph nodes) and presence of untargeted tumors after each TACE session.

4.2.2.1 Assessment of tumor response according to RECIST

At the first follow-up visit, 29 patients showed partial response, 79 had stable dis-

RECIST	N	%	N	%	Change	N	%	Change

ease, while 11 progressed. At the second follow-up visit, 32 patients showed partial response, 74 remained stable and 12 showed progressive disease. At the last follow-up, 28 patients showed partial response, 59 had stable disease, while 32 progressed. **Table 10** shows the distribution of patient tumor response according to RECIST during each study time point, as well as the percent change from baseline in the number of responders per each RECIST category. **Figure 7** shows a spaghetti plot of the fitted RECIST values over time (visit) for each patient. A Spearman's correlation coefficient was calculated to assess the relationship between RECIST and CTP score at each visit. There was no statistically significant correlation between RECIST and CTP at the first follow-up (r_s = 0.01, p = 0.88, Bonferroni adjusted), 2nd follow-up (r_s = -0.07, p = 0.44, Bonferroni adjusted) or last follow-up (r_s = -0.10, p = 0.25, Bonferroni adjusted).

					(%)			(%)
	1st follow-up		2nd follow-up			Last follow-up		
SD	79	66.39	74	62.18	-6.33	59	49.58	-11.13
PR	29	24.37	32	26.89	10.34	28	23.53	14.90
PD	11	9.24	12	10.08	9.09	32	26.89	246.32

Table 11. Patient tumor response to TACE according to RECIST, over time (study follow-up visits). Percent change indicates the relative change in the number of patients for each visit, compared to the 1st follow-up visit, by RECIST category.

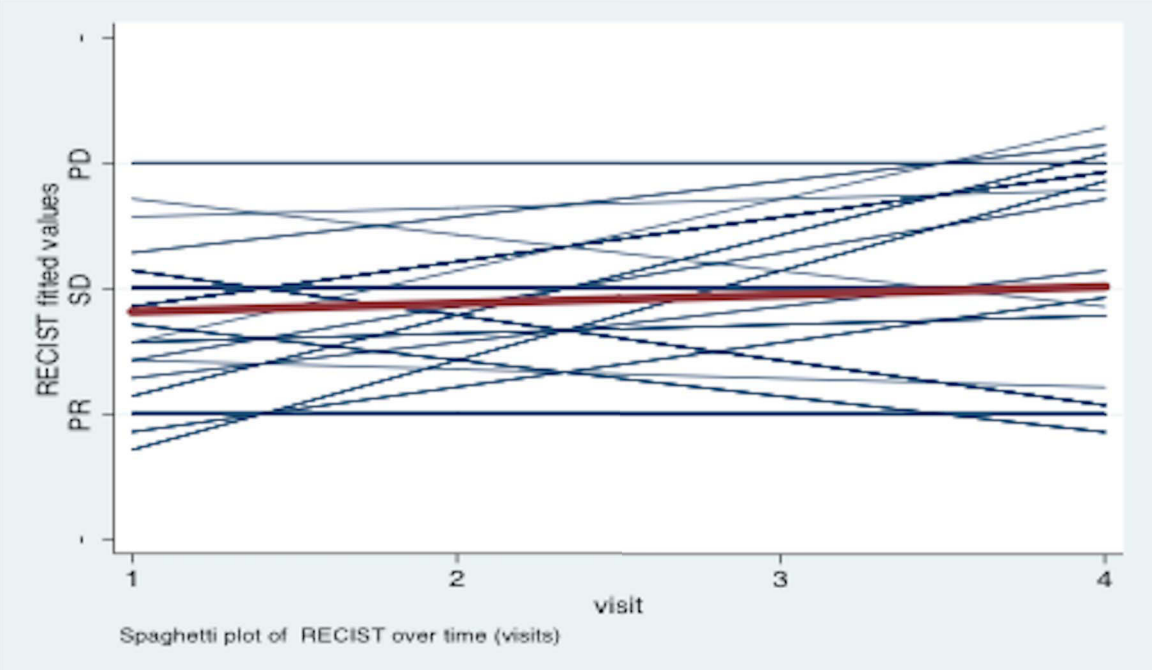

Figure 7. Spaghetti plot of fitted RECIST response (fitted values) by visit for each patient over the study period of 3 consecutive visits and the last before study exit visit. The red line represents the overall fitted regression line of RECIST on visit number.

4.2.2.2. Tumor response according to mRECIST

Complete tumor response according to mRECIST was achieved in 25 patients at the first follow-up visit. In addition, 25 patients showed partial response, 58 had stable disease and 11 patients showed progressive disease. At the end of the study period 9.24% of patients demonstrated complete response, while 41.18% demonstrated stable disease. **Table 12** shows the distribution of tumor response according to mRECIST during each study time point, as well as the percent change from baseline in the number of responders per each mRECIST category as compared to the 1st visit. **Figure 9** shows a spaghetti plot of the fitted RECIST values over time (visit) for each patient.

A Spearman's correlation coefficient was calculated to assess the relationship between mRECIST and CTP score at each visit. There was no statistically significant correlation between mRECIST and CTP at the first follow-up ($r_s = 0.01$, $p = 0.86$, Bonferroni adjusted), 2nd follow-up ($r_s = 0.05$, $p = 0.58$, Bonferroni adjusted), or a last follow-up ($r_s = 0.17$, $p = 0.05$, Bonferroni adjusted).

Table 12. Patient response to TACE according to mRECIST at each study visit. N indicates number of patients and % indicates percentage of patients for each mRECIST category at each time point. Percent change indicates the relative change in the number of patients at each visit compared to the first visit, by mRECIST category.

mRECIST	N	%	N	%	Change (%)	N	%	Change (%)
	1st follow-up		2nd follow-up			Last follow-up		
SD	58	48.74	58	48.74	0	49	41.18	-15.52
PR	25	21.01	33	27.73	32	27	22.69	8.00
CR	25	21.01	17	14.29	-32	11	9.24	-56.00
PD	11	9.24	10	8.4	-9.09	32	26.89	190.91

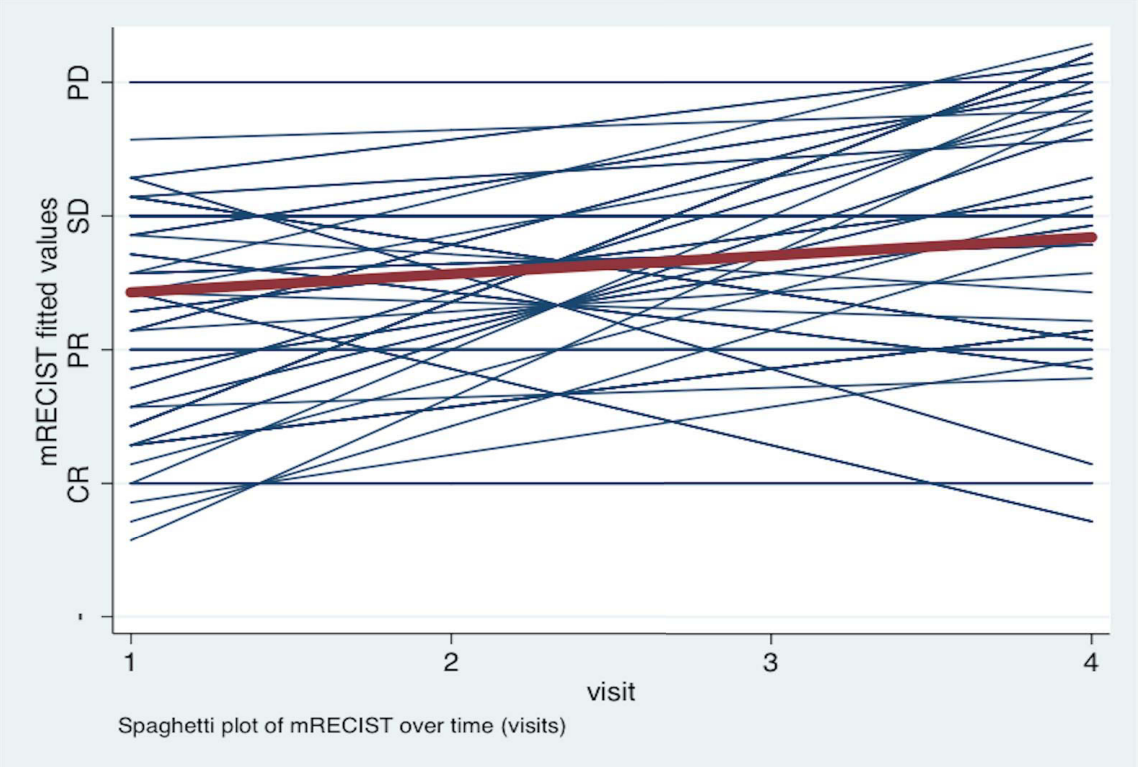

Figure 8. Spaghetti plot of fitted mRECIST response (fitted values) by visit for each patient over the study period of 3 consecutive visits and the last before study exit visit. The red line represents the overall fitted regression line of mRECIST on visit.

4.2.2.3. Tumor Response According To Tumor Size Change

The mean longest baseline axial tumor diameter was 7.76 cm (SD=3.62, range 2-16 cm). At 1st follow-up, the mean longest axial diameter was 7.09 cm (SD=3.92; range: 0.8-19 cm, p=0.20, unpaired t-test, compared to baseline).). At the 2nd follow-up, the mean longest axial diameter further decreased to 6.95 cm (SD=3.95; range" 0.8-19 cm, p=0.12, unpaired t-test, compared to baseline).). At the final follow-up, mean axial tumor diameter was 6.74 (SD=3.87; range=0.8-19 cm, p=0.05, unpaired t-test, compared to baseline). **Table 12** demonstrates the summary statistics for longest axial tumor size and percent change in longest axial tumor size over the study period. Compared to baseline, there is a statistically significant change in longest uni-dimensional tumor size at 6 months (p=0.03, paired t-test). Compared to percent change in longest uni-dimensional tumor size between baseline and 1st visit, there is a statistically significant change between 2nd visit and baseline (p= 0.01, paired t-test), as well as between baseline and last visit (p=0.01, paired t-test). **Figure 9** depicts a waterfall plot of the tumor size percent change for each individual and visit. **Figure 10** depicts the longest uni-dimensional tumor size measurements over time (study visits).

Table 13. Descriptive statistics for longest axial tumor size and percent change in longest axial tumor size at each study visit. N indicates number of patients and parentheses indicate percentage of patients evaluated at each time point. N=number of observations, SD=standard deviation, min=minimum, max=maximum. P-values are recorded for unpaired t-test comparisons for tumor size and paired t-test comparisons for change (%) in tumor size, in regards to baseline values.

Variable	**Visit**	**N**	**Mean**	**SD**	**Min**	**Max**	**P-value**
Tumor Size (cm)	Baseline	94	7.76	3.62	2	16	
	1st visit	117	7.09	3.92	0.8	19	0.20
	2nd visit	116	6.95	3.95	0.8	19	0.12
	Last visit	117	6.74	3.87	0.8	19	0.05
Change (%) in Tumor Size from Baseline	1st visit	92	-19.95	98.85	-375	92	
	2nd visit	92	-14.90	93.05	-275	92	0.01
	Last visit	92	-8.89	83.33	-244	92	0.02

Figure 9. Waterfall plot of percent change in longest tumor uni-dimensional size for each study visit compared to baseline tumor size measurement, by patient.

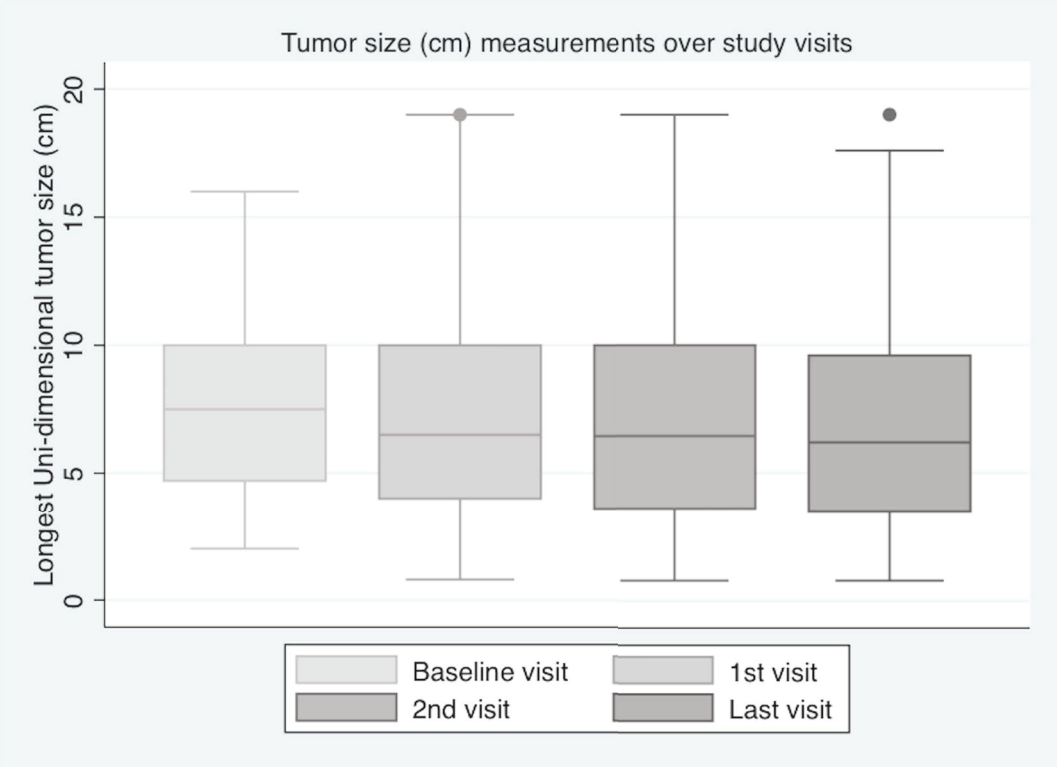

Figure 10. Longest tumor uni-dimensional size measurements over study visits (p-value=0.01, multivariate F-test of means).

4.3. Analysis Of Survival Estimates

Mean overall survival was 18.85 months and median 14 months (range: 1-148 months). During the study follow-up period, 95 of the 119 patients died. **Figure 11**. displays the Kaplan-Meyer estimates of the overall probability of survival for the entire cohort.

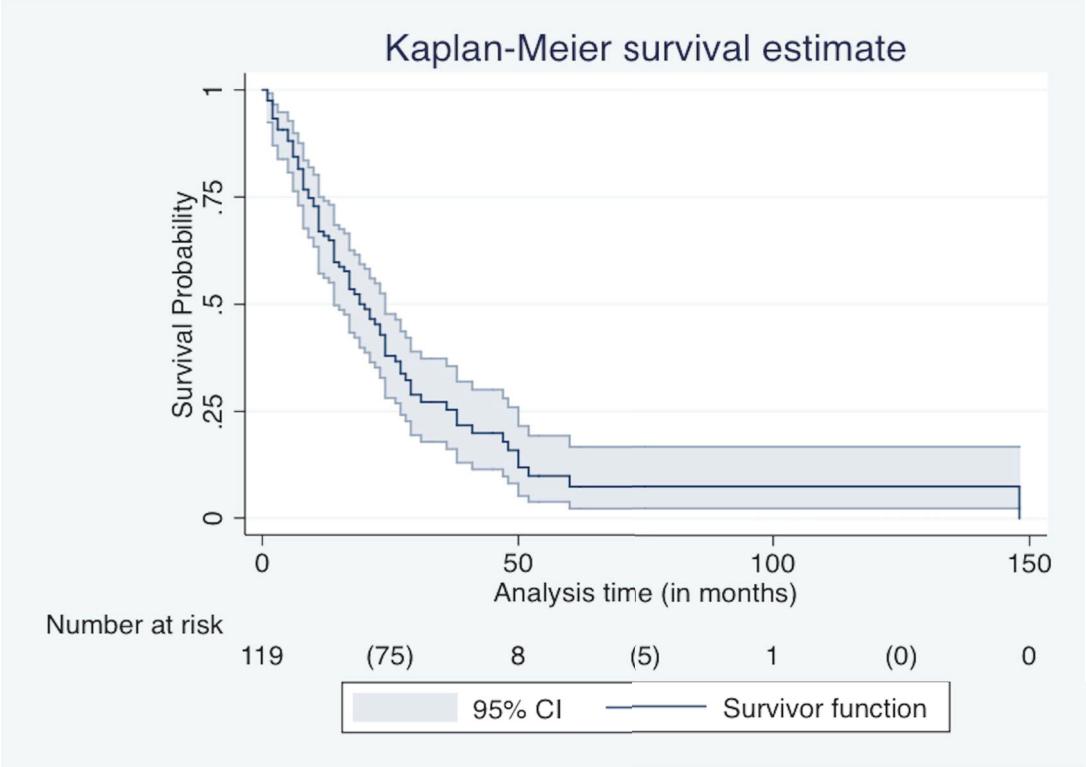

Figure 11. Kaplan-Meyer estimates of the overall probability of survival for the entire cohort of patients with unresectable HCC that were treated with TACE. Survival estimates are calculated in months from the time of the first clinical visit to the Interventional Radiology Center till study exit.

There was no statistically significant difference in survival estimates among age groups (p-value=0.30, log-rank test). There was no statistically significant difference in survival estimates between male and female patients (p-value= 0.97, log-rank test). Similarly, there was no statistically significant difference in survival estimates among ethnicities (p=0.57, log-rank test**.)**. There was no statistically significant difference in survival estimates among patients with different etiologies of hepatic cirrhosis (p=0.21, log-rank test). However, there were statistically significant differences in survival estimates among baseline ECOG performances with higher survival in ECOG 0 as compared to 1 and 2 (p=0.006, log-rank test, **Figures 12 and 13**). There were statistically significant differences in survival estimates by baseline CTP, with higher survival in CTP A as compared to CTP B and C (p-values=0.005, log-rank test, **Figures 14 and 15).** There was no statistically significant difference in survival estimates among patients with different 1st RECIST responses (p=0.30, log-rank test). There was no statistically significant difference in survival estimates among patients with different 1st mRECIST responses (p=0.38, log-rank test**.**). There was a statistically significant difference in survival estimates among baseline tumor size groups, with higher survival in patients with baseline tumor size < 5 cm (p-values=0.002, log-rank test, **Figures 16 and 17).** There was no statistically significant difference in survival estimates among patients with and without extrahepatic metastases (p=0.35, log-rank test). There was a statistically higher survival among patients without, as compared to with, baseline portal vein thrombosis (p-values=0.005, log-rank test, **Figure 18). Table 13** shows the time at risk,

number of observations, median and interquartile range of survival estimates among patients by baseline variables. P-values indicate significance by the log-rank test.

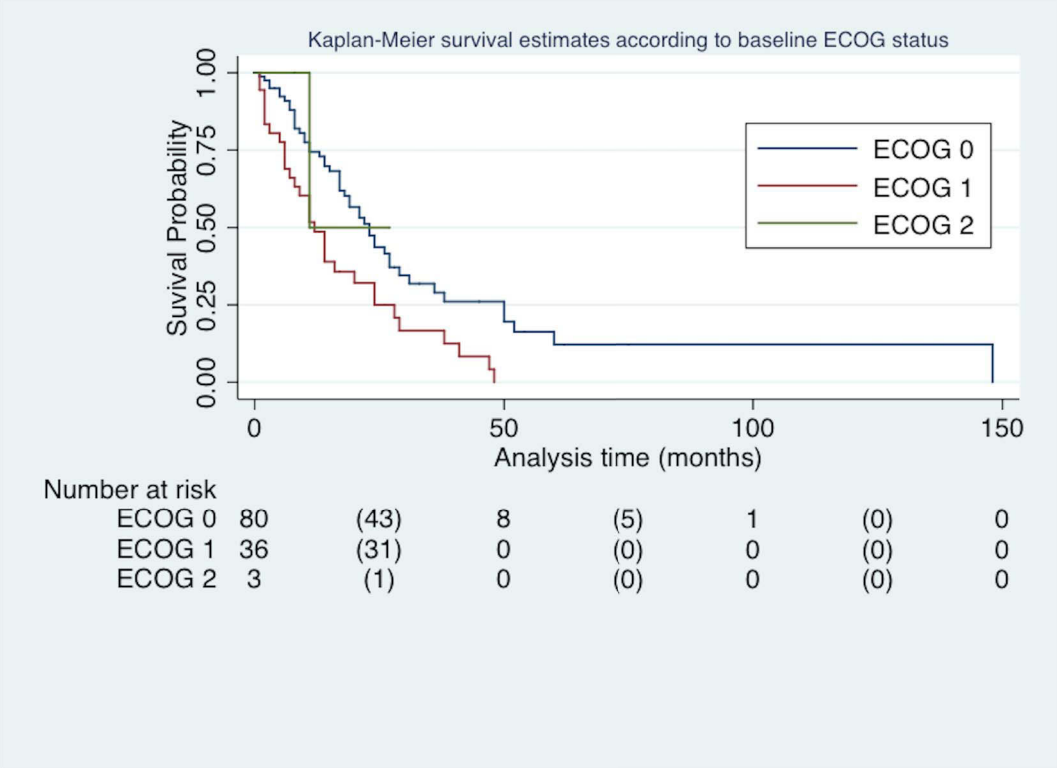

Figure 12. Kaplan Meier survival estimates according to baseline ECOG performance status. (Log rank p-value=0.006)

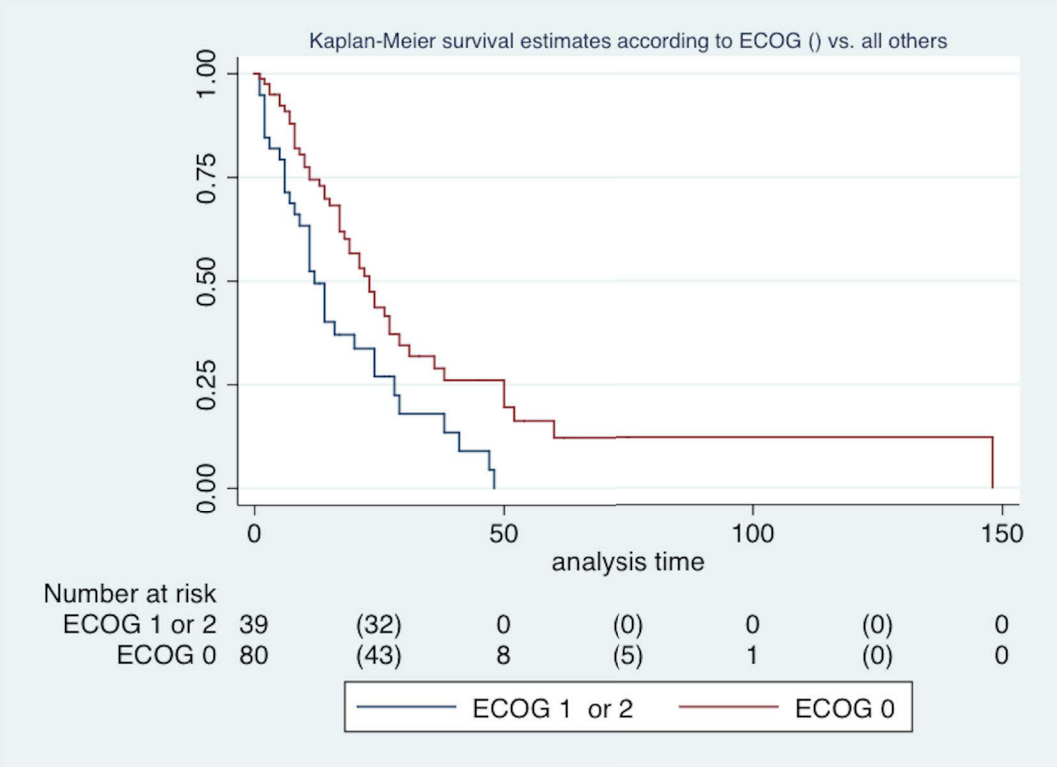

Figure 13. Kaplan Meier survival estimates according to baseline ECOG performance status (0 vs. all others). (Log rank p-value=0.0035)

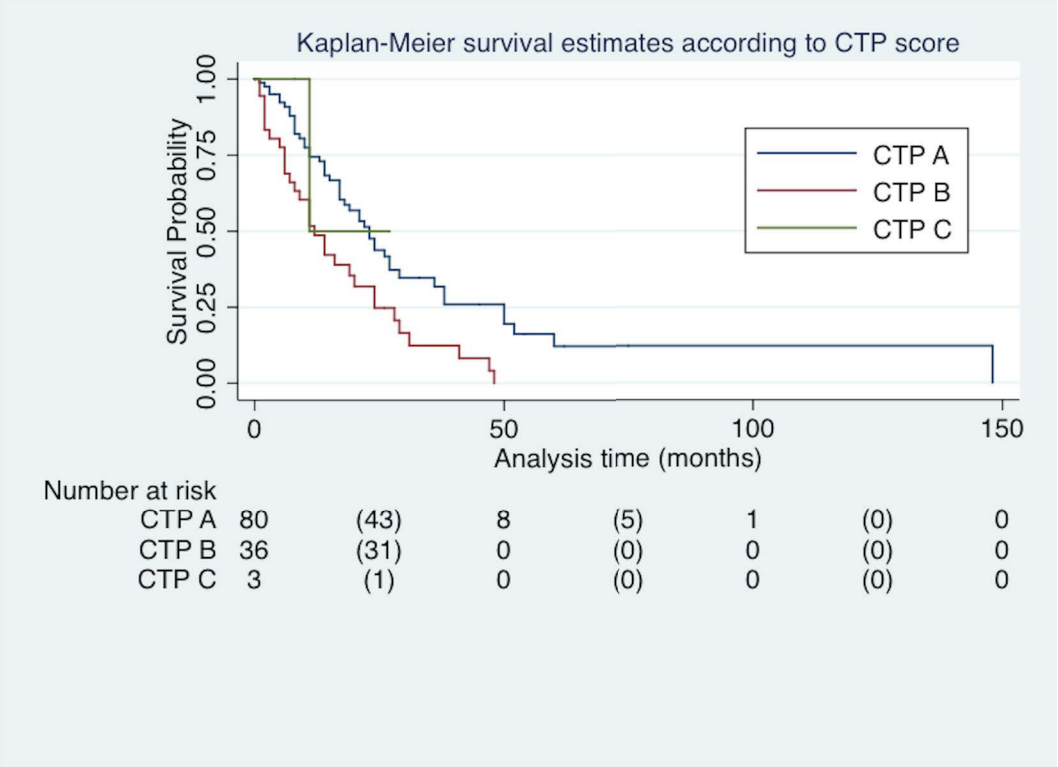

Figure 14. Kaplan Meier survival estimates according to baseline CTP scores. (Log rank p-value=0.005)

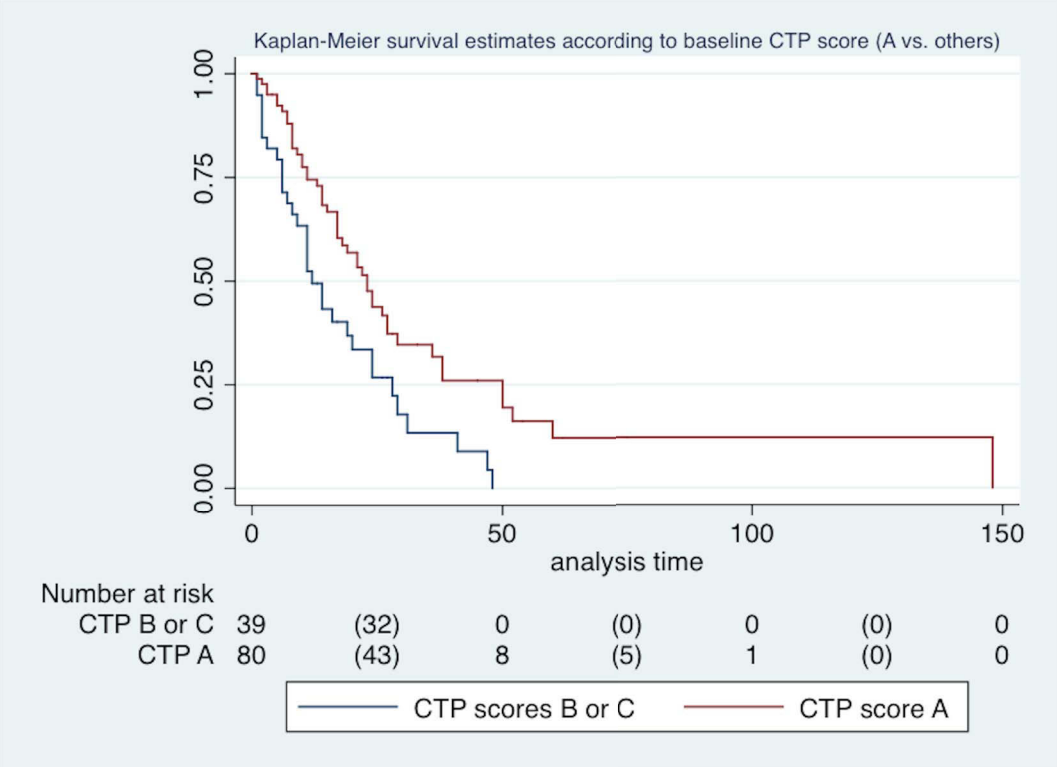

Figure 15. Kaplan Meier survival estimates according to baseline CTP scores (A vs. all other scores). (Log rank p-value=0.034)

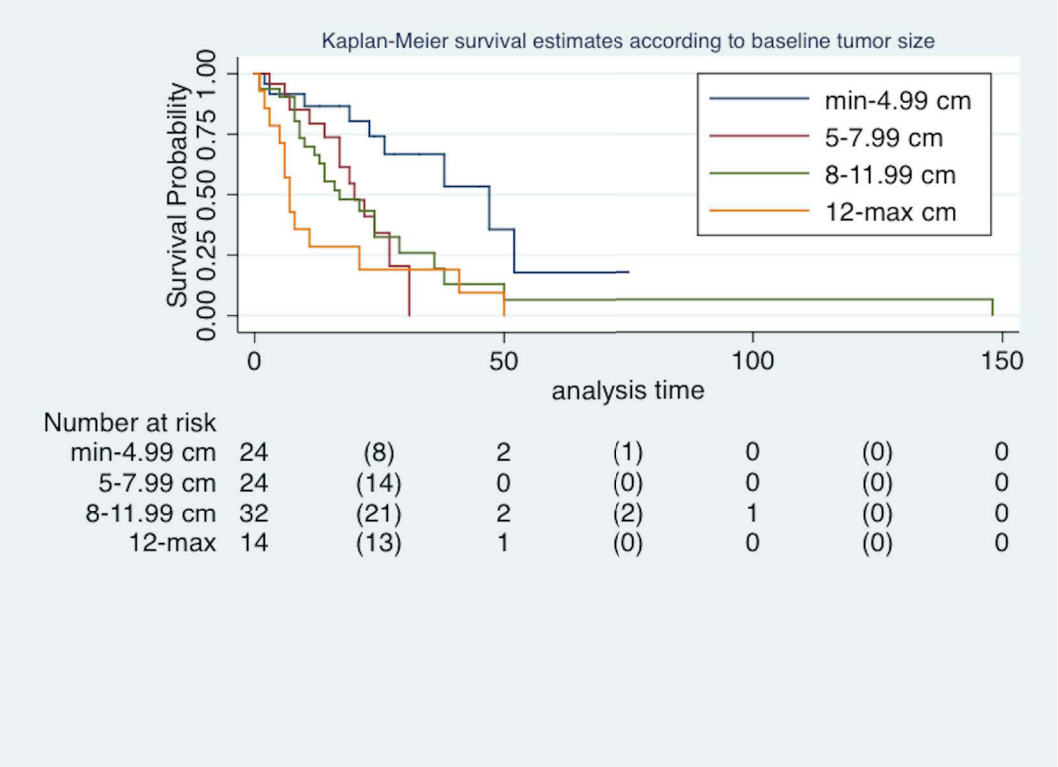

Figure 16. Kaplan Meier survival estimates according to baseline longest uni-dimensional tumor size. (Log rank p-value=0.002)

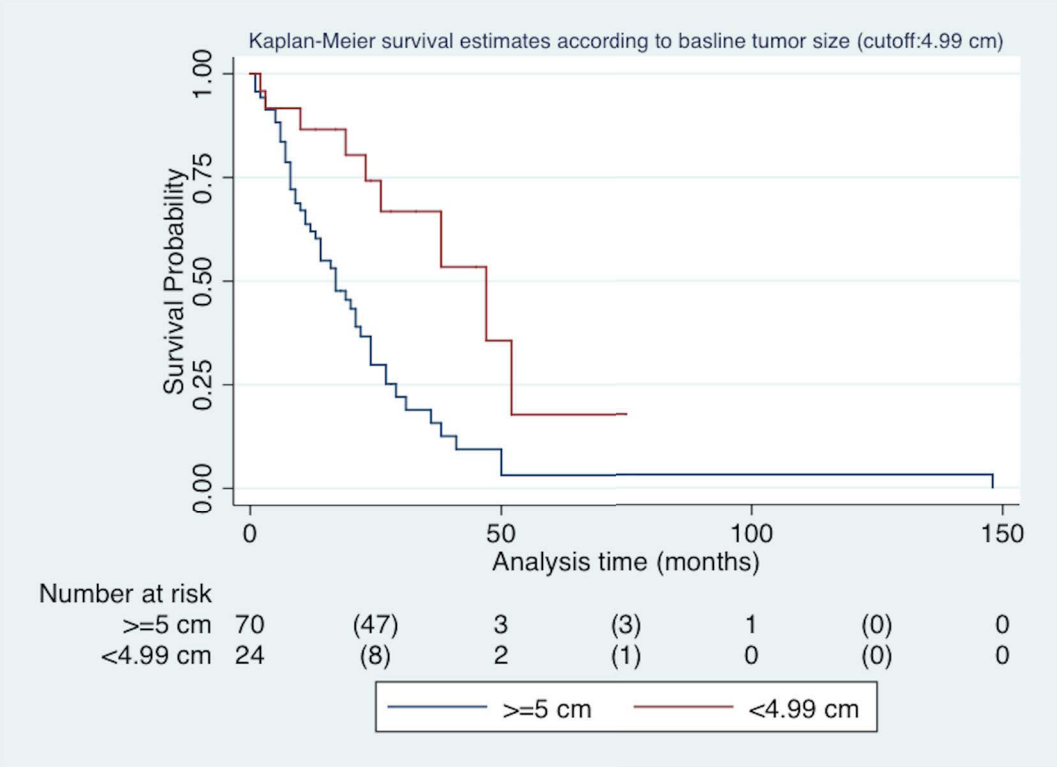

Figure 17. Kaplan Meier survival estimates according to baseline longest uni-dimensional tumor size cutoff of 4.99 cm. (Log rank p-value=0.0017)

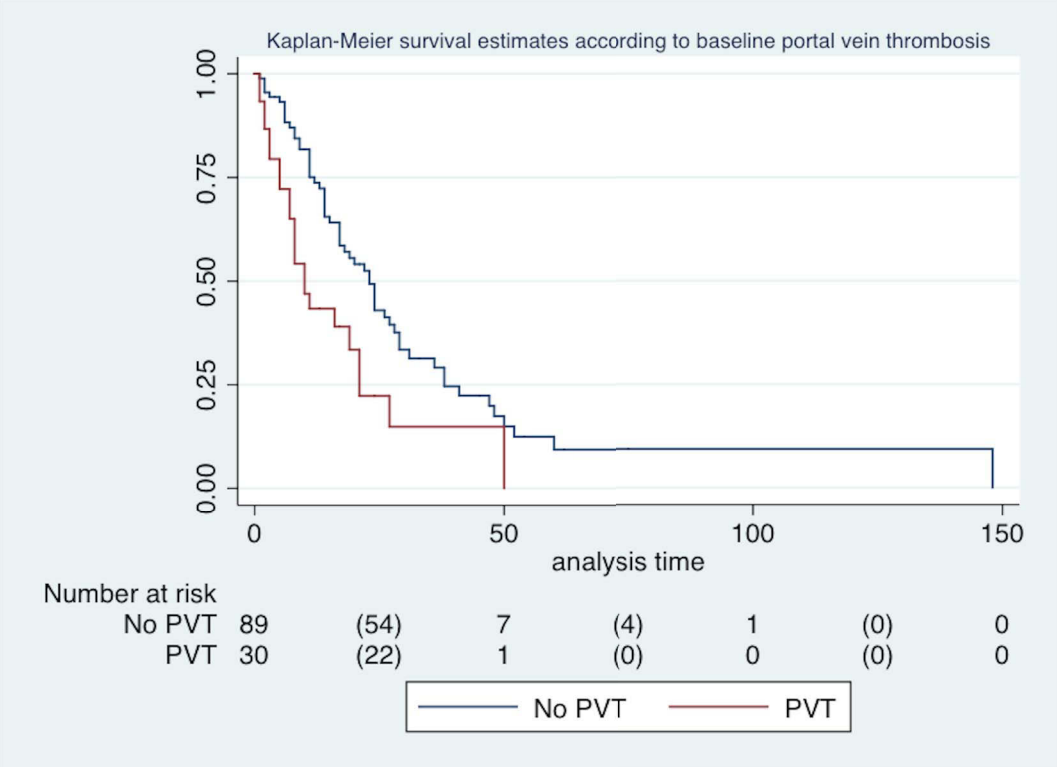

Figure 18. Kaplan Meier survival estimates according to baseline portal vein thrombosis. (Log rank p-value=0.0037)

Table 14. Time at risk, number of observations, median and interquartile range of survival estimates among patients by baseline variables. P-values indicate significance by the log-rank test.

		Time at Risk	Incidence Rate	N	25%	50%	75%	p-value
Gender	Male	1774	0.036	99	8	19	38	0.979
	Female	469	0.031	20	14	23	26	
Age (years)	16-37	132	0.007	6	14			0.368
	38-59	902	0.036	47	9	18	29	
	60-80	1081	0.037	59	10	21	36	
	81-102	127	0.031	6	8	11		
Ethnicity	Caucasian	1409	0.038	79	10	21	31	0.241
	African American	356	0.039	22	8	19	47	
	Asian	1169	0.035	9	14	18	50	
	Other	307	0.016	8	15	36	148	
ECOG status	0	1666	0.028	86	12	23	50	**0.006**
	1	531	0.058	44	5	11	24	
	2	46	0.021	3	11	11	.	
Cirrhosis	Unknown/ cryptogenic	725	0.040	34	8	14	38	0.215
	Hepatitis B	415	0.026	23	11	27	50	
	Hepatitis C	510	0.043	35	11	21	26	
	Hepatitis B and alcohol	85	0.058	5	3	6	36	
	Other*	508	0.025	22	14	24	47	
Baseline CPT score	A	1668	0.028	80	11	23	50	**0.015**
	B	529	0.058	36	6	12	24	
	C	46	0.021	3	11	11	.	
1st RECIST	PR	660	0.028	29	10	21	50	0.300
	SD	1365	0.041	79	9	17	29	

	PD	218	0.022	11	11	48	48	
1st mRECIST	CR	473	0.040	25	10	15	38	0.380
	PR	590	0.027	25	11	23	52	
	SD	962	0.041	58	9	17	27	
	PD	218	0.023	11	11	48	48	
Baseline PVT	No	1883	0.031	89	12	23	38	**0.003**
	Yes	360	0.061	30	5	10	21	
Baseline tumor size (cm)	2-4.99	561	0.016	24	23	47	52	**0.002**
	5-7.99	361	0.038	24	14	20	27	
	8-11.99	640	0.035	32	9	17	36	
	12-16	179	0.072	14	5	7	21	
Extrahepatic metastases								0.351
	No	1515	0.040	91	8	18	38	
	Regional lymph nodes	404	0.024	14	12	21	36	
	Distant	324	0.027	14	13	29	50	

*Includes combination of alcoholic and HCV cirrhosis, combination of alcoholic and HBV/HCV cirrhosis, hemochromatosis, hemochromatosis and HBV, hemochromatosis and HCV, non-alcoholic steatohepatitis, or none

4.4. Cox Regression Analysis

4.4.1. Univariate Cox Regression Analysis using Baseline (Time-Independent Variables)

Time-independent Cox regression analysis included a univariate Cox regression analysis to evaluate the association between each baseline covariate and the hazard for death.

Univariate Cox regression analysis showed that the hazard of death was higher in older patients compared to younger ones (for age group 37-59 years compared to less than 37 years, HR=5.49, 95% CI: 0.75-40.22, p=0.09; for age group 60-80 years compared to less than 37 years, HR= 5.30, 95% CI: 0.73-38.61, p=0.1; for age group older than 80 years compared to less than 37 years, HR=4.83, 95% CI: 0.54, 43.36, p=0.16). The hazard of death was significantly higher for patients with ECOG status of 1 or 2, as compared to 0 (HR =1.96, 95% CI: 1.23-3.11, p=0.004); CTP categorical score B as compared to A (HR= 2.07, 95% CI: 1.30, 3.28, p= 0.002); larger tumors compared to those < 5 cm (5-7.99 cm, HR=2.70, 95% CI=1.14, 6.37, p=0.024; 8-11.99 cm, HR= 2.60, 95% CI= 1.19, 5.99, p< 0.016; larger than 12 cm, HR= 4/85, 95% CI: 2.05-11.49, p<0.0001); and presence of portal vein thrombosis at baseline (HR=2.074, 95% CI: 1.25, 3.43, p=0.004). Univariate Cox regression analysis showed that the hazard of death was slightly lower in patients with HBV (HR=0.59, 95% CI: 0.28-1.16, p=0.16) and in patients with cirrhosis of other etiology, including hereditary hemochromatosis

(HR=0.56, 95% CI: 0.27-1.13, p=0.11). **Table 14** provides the estimated hazard ratio (HR) for death for each baseline covariate, the corresponding 95% confidence interval (lower and upper CI) and p-value for the corresponding Wald test of the univariate Cox regression analyses. All of the statistically significant variables, defined as those with a Wald test p-value of less than 0.20 (Greenland, 1989) from the univariate analysis were subsequently included in the multivariable model in order to assess the combined effect of all risk factors of early mortality and to eliminate the effect of confounding factors. Note that in addition to the cutoff value of $p<0.20$, clinical criteria were also employed for multivariable modeling.

Table 15. Univariate Cox regression analysis of baseline covariates and hazard ratio for death during the study period of patients with unresectable HCC treated with TACE. Values are unadjusted. HR=Hazard Ratio, LCI=lower 95% CI, UCI=Upper 95% CI, P-value=Wald test P-value.

	HR	z	P-value	LCI	UCI
Age (years)					
16-37.5	1				
38-59	5.49	1.67	**0.09**	0.75	40.22
60-80	5.30	1.65	**0.10**	0.73	38.61
>80	4.83	1.41	**0.16**	0.54	43.36
Gender					
Male	1				
Female	1.01	0.03	0.98	0.57	1.80
Ethnicity					
Caucasian	1				
African American	1.07	0.23	0.82	0.59	1.93
Asian	0.94	-0.14	0.89	0.40	2.19
Other	0.47	-1.43	**0.15**	0.17	1.32
Hepatic Cirrhosis					
HCV	1				
Unknown/cryptogenic	0.95	-0.16	0.88	0.54	1.70
HBV	0.59	-1.42	**0.16**	0.28	1.22
HBV and Alcohol	1.32	0.56	0.58	0.49	3.57
Other*	0.56	-1.61	**0.11**	0.27	1.13
Baseline ECOG score					
0	1				
1 or 2	1.96	2.88	**0.004**	1.23	3.10
Extrahepatic Metastases					
None	1				
Regional Lymph nodes	0.70	-0.97	0.33	0.35	1.43
Distant Metastasis	0.66	-1.17	0.24	0.33	1.33
Baseline Portal Vein Thrombosis					
No	1				
Yes	2.074	2.840	**0.004**	1.254	3.430
Baseline Tumor Burden					

Single Tumor	1				
2-3 tumors	0.91	-0.24	0.81	0.44	1.91
>3 tumors	0.91	-0.42	0.68	0.57	1.45
Baseline Tumor Size (cm)					
2-4.99	1				
5-7.99	2.70	2.27	**0.023**	1.14	6.37
8-11.99	2.60	2.40	**0.016**	1.19	5.69
12-16	4.85	3.59	**<0.0001**	2.05	11.49
Baseline CTP score					
A	1				
B	2.07	3.07	**0.002**	1.30	3.28
C	0.75	-0.29	0.77	0.10	5.45
1st mRECIST					
CR	1				
PR	0.71	-0.99	0.32	0.36	1.40
SD	1.07	0.25	0.80	0.62	1.86
PD	0.58	-1.07	0.29	0.22	1.57
1st RECIST					
PR	1				
SD	1.35	1.11	0.27	0.79	2.30
PD	0.76	-0.54	0.59	0.28	2.06

*Includes combination of alcoholic and HCV cirrhosis, combination of alcoholic and HBV/HCV cirrhosis, hemochromatosis, hemochromatosis and HBV, hemochromatosis and HCV, non-alcoholic steatohepatitis, or none

4.4.2. Assessment of PH assumption for baseline covariates

To visually inspect the proportional hazards assumption, "Stphplot" plots –ln (-ln (survival)) or “complementary log-log” survival curves for each variable versus ln (time) were constructed. The proportional hazards assumption is not violated when the curves are parallel. **Figure 19** shows the complementary log-log survival plot of baseline portal vein thrombosis versus ln (time), where the PH assumption seems not to be violated. **Figure 20** shows the complementary log-log survival plot of baseline ECOG performance status versus ln (time), where again, the PH assumption seems not to be violated. **Figure 21** shows the complementary log-log survival plot of baseline tumor size versus ln(time). The two lines (for baseline tumor size >5 cm and =<4.99 cm) do not run parallel over time.

Following, we generated plots of the generalized linear regression of the scaled Schoenfeld residuals of selected covariates on time and employed the Schoenfeld test (stphtest), to investigate the linear trend between the Schoenfeld residuals and “log” survival time. **Figure 22** shows the generalized linear regression of the scaled Schoenfeld residuals of tumor size on time (p=0.21 for the global test). **Table 15** provides the Schoenfeld test p-values (prob>chi2), correlation coefficient (r), the chi2 test values and degrees of freedom (df) for all tested covariates. The global test output from the Schoenfeld test is not statistically significant, indicating an absence of evidence to contradict the proportionality assumption. The test output for African American ethnicity is statistically significant (p=0.04), indicating that proportionality is violated in this

predictor. Stratification according to ethnicity may be suggested as an alternative to bypass this violation.

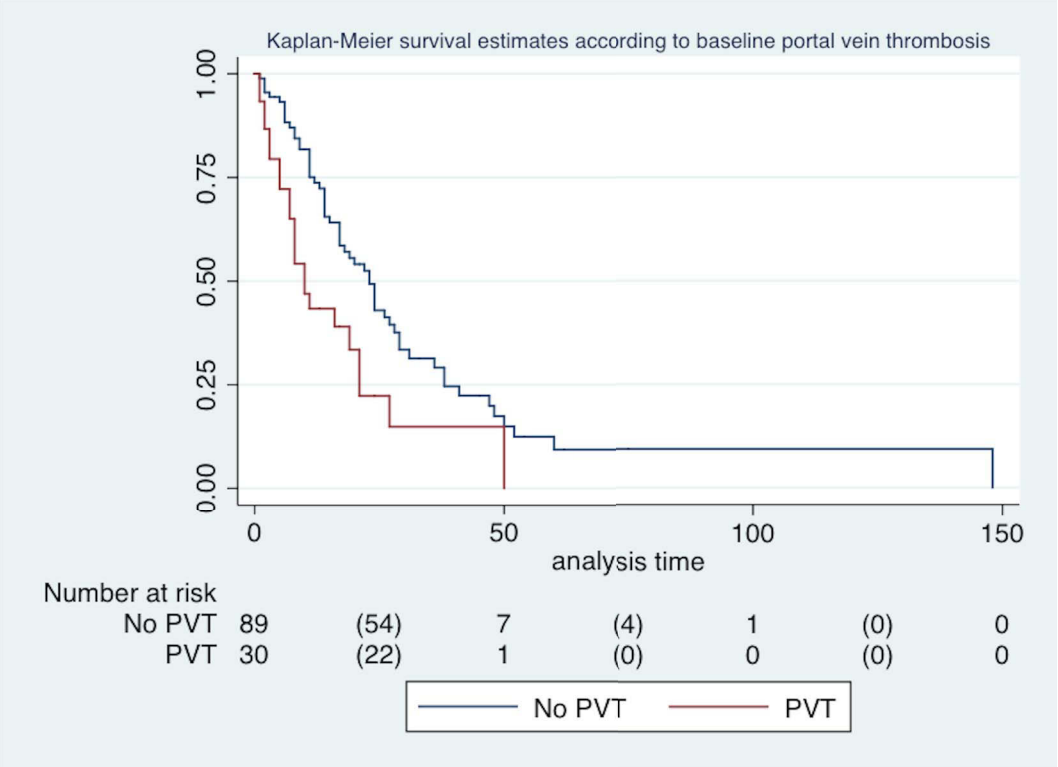

Figure 19. Complementary log-log survival plot of baseline portal vein thrombosis versus ln (time). The two lines run parallel and the PH assumption seems not to be violated.

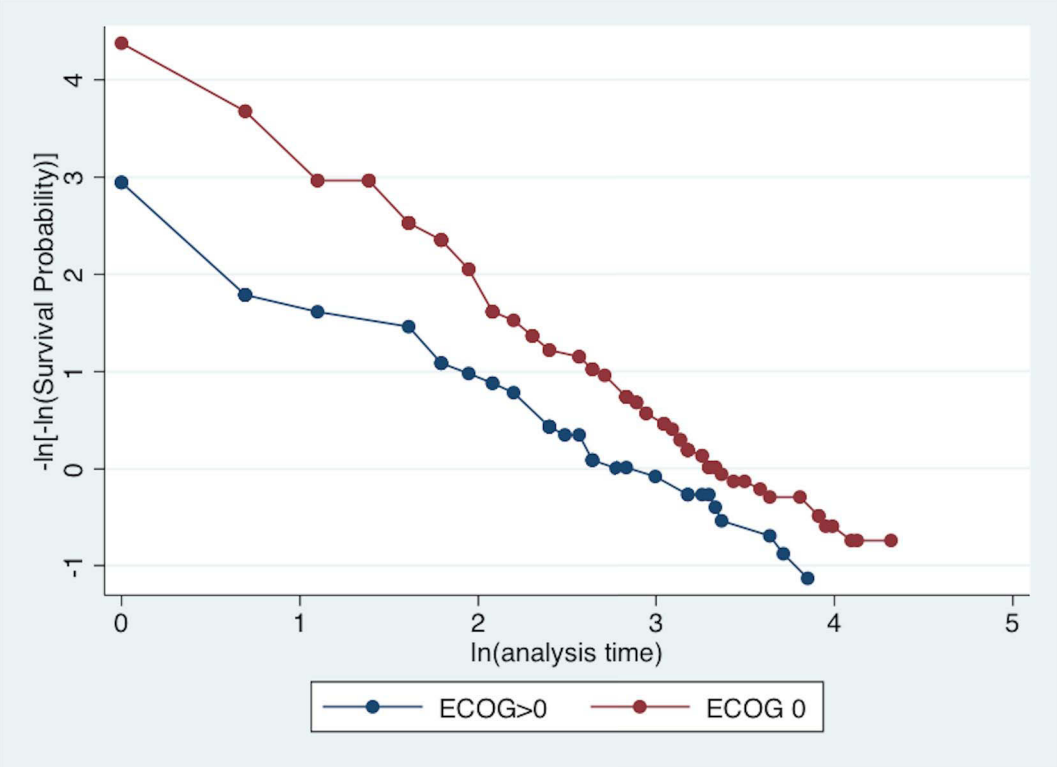

Figure 20. Complementary log-log plot of baseline ECOG status versus ln (time). The two lines run parallel and the PH assumption seems not to be violated.

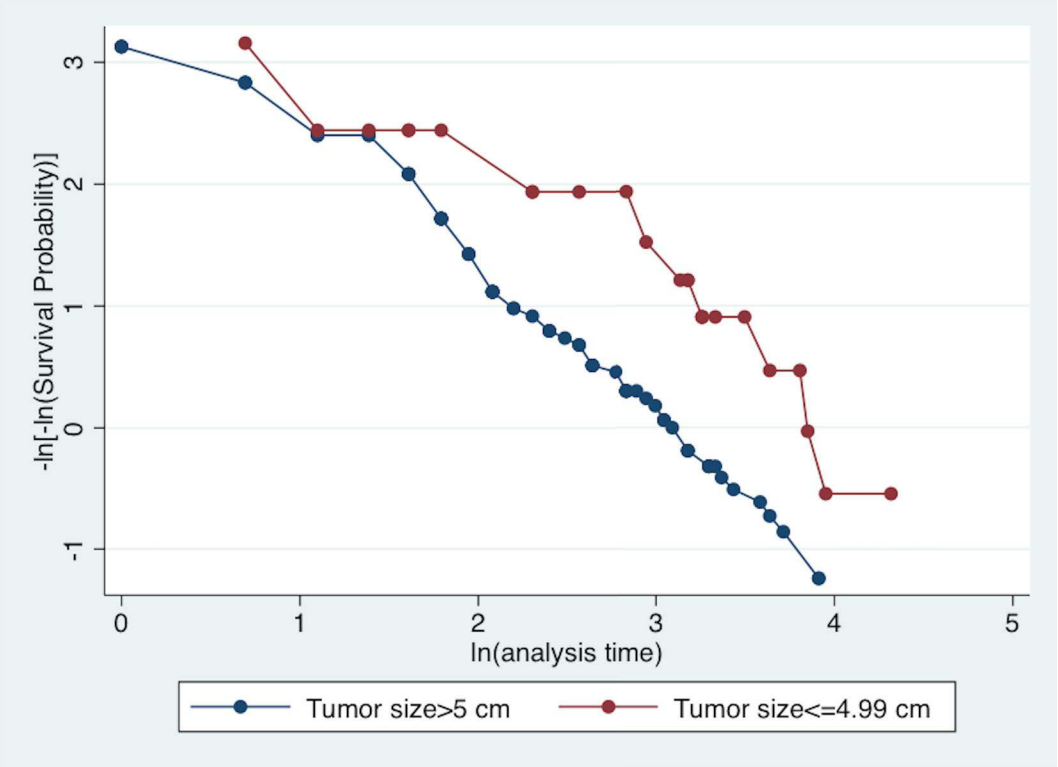

Figure 21. Complementary log-log plot of baseline tumor size versus ln (time). The PH assumption may be violated, as the two lines do not run parallel over time.

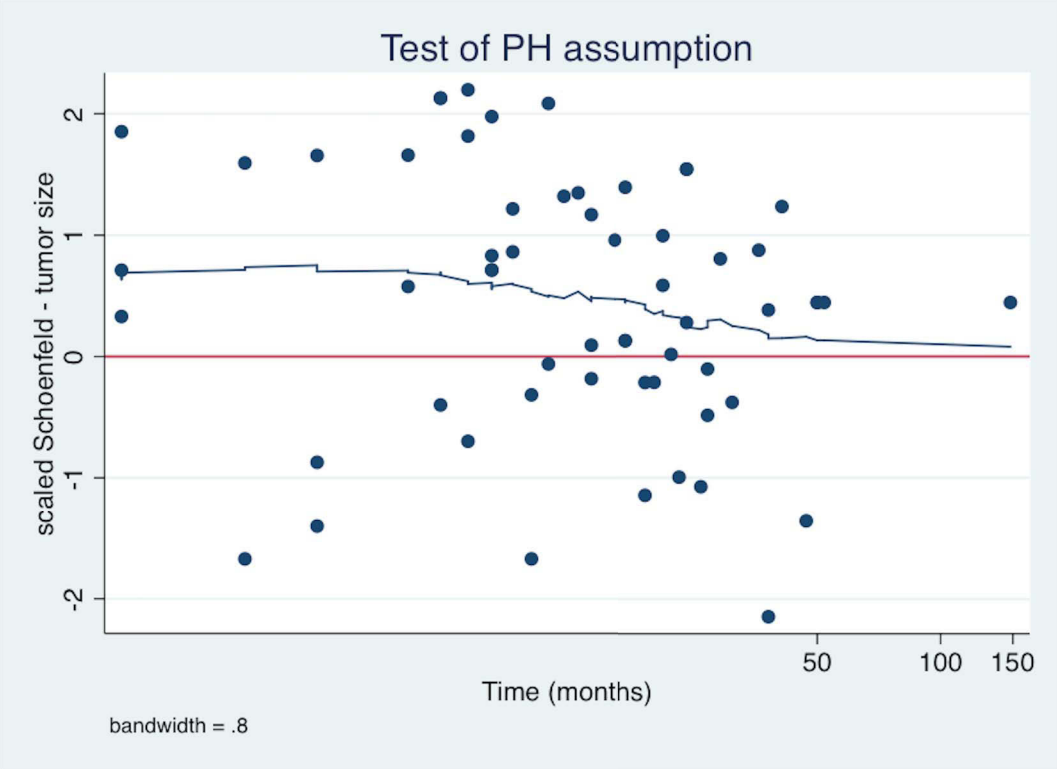

Figure 22. Graph of generalized linear regression of the scaled Schoenfeld residuals of baseline tumor size on time. The red line is a horizontal reference line at y=0. The curved blue line is a LOWESS fit (locally weighted scatterplot smoothing) curve of the residuals on time, to facilitate pattern detection.

Table 16. Schoenfeld tests of proportional hazards assumption for covariates included in the multivariable Cox regression analysis**.** Time is expressed as log survival time. Prob>chi^2=Schoenfeld test p-value, rho= correlation coefficient, df=the chi2 test values and degrees of freedom for all tested covariates.

Time: Log (t)	**rho**	**chi2**	**df**	**Prob>chi^2**
Age (years)				
16-37.5 (base)	.	.	1	.
38-59	-0.036	0.07	1	0.787
60-80	-0.033	0.06	1	0.801
>80	-0.212	2.61	1	0.106
Ethnicity				
Caucasian (base)	.	.	1	.
African American	-0.233	4.28	1	**0.04**
Asian	0.006	0	1	0.950
Other	-0.10	1.08	1	0.300
Baseline Portal Vein Thrombosis				
No (base)	.	.	1	.
Yes	-0.122	1.16	1	0.281
Baseline CTP score				
A (base)	.	.	1	.
B	-0.117	0.77	1	0.380
C	0.032	0.08	1	0.773
Hepatic Cirrhosis				
HCV (base)	.	.	1	.
Unknown/cryptogenic	-0.1702	2.24	1	0.13
HBV	-0.119	1.06	1	0.30
HBV and Alcohol	-0.059	0.27	1	0.60
Other*	0.015	0.01	1	0.90
Baseline ECOG score				
0 (base)	.	.	1	.
1	0.008	0	1	0.44
Baseline Tumor Size (cm)				
2-4.99 (base)	.	.	1	.
5-7.99	0.160	1.76	1	0.18

8-11.99	0.160	1.61	1	0.20
12-16	-0.012	0.01	1	0.91
Global test		**21.37**	**17**	**0.21**

*Includes combination of alcoholic and HCV cirrhosis, combination of alcoholic and HBV/HCV cirrhosis, hemochromatosis, hemochromatosis and HBV, hemochromatosis and HCV, non-alcoholic steatohepatitis, or none

4.4.2. Time-independent Multivariable Cox Regression Analysis

Time-independent multivariable Cox regression analysis was performed to evaluate how baseline covariates jointly affect patient survival. Statistically significant covariates ($p<0.2$) from the univariate Cox regression models were incorporated into the multivariable Cox regression analysis. **Table 16** shows the results of the multivariable Cox regression analysis of baseline covariates on the effect of relative hazard of death for the study cohort **(Model A)**.

Given that the goal of the multivariable Cox model is to assess the effect of TACE on overall survival, while controlling for putative confounding variables, potential confounders were examined by using the change-in-estimate method and by comparing unadjusted and adjusted coefficients of HR. In this method, a potential confounder was included in the model if it changed the coefficient of the primary exposure variable (HR) by 10 percent. This approach is consistent with the definition of confounding, with confounding being present if the unadjusted effect differs from the effect adjusted for putative confounders (Rothman, Greenland, & Lash, 2008). **Table 17** shows the comparison of unadjusted and adjusted hazard ratios of death for baseline variables tested in univariate and multivariable (Model A) Cox regression analyses. The variables of age, ethnicity, ECOG status, etiology of cirrhosis and CTP score had more than 10% in change between unadjusted and adjusted effect estimates, indicating that these are confounders of the relationship between the predictors of portal vein thrombosis and tumor size on overall survival.

Table 18 shows the results of the multivariable Cox regression analysis of baseline covariates on the effect of relative hazard of death, stratified by ethnicity **(Model B)**. Stratification by ethnicity was performed after testing for PH assumption using the Schoenfeld (stph) test, where it was observed that PH proportionality for African American ethnicity was violated (global test, p-value=0.04). The multivariable Cox regression analysis shows that the presence of portal venous thrombosis (HR=2.77, 95% CI=1.43-5.47, p=0.002) and baseline tumor size less than 5 cm (5-7.99 cm: HR=3.62, 95% CI=1.43-9.15, p=0.007; 8-11.99 cm: HR=3.24, 95% CI=1.30-8.08, p=0.011; >12 cm: HR=9.51, 95% CI=2.88-31.41, p<0.0001) are independent statistically significant predictors of overall survival. After regrouping patients according to the cut-off value of 5 cm in uni-dimensional tumor size, patients with smaller tumors had a statistically significant higher hazard of death (HR=3.60, 95% CI=1.60-8.09, p=0.002). Stratified by ethnicity, the hazard ratio of mortality for patients with unresectable HCC and portal venous thrombosis before 1st TACE is 2.68 times higher (95% CI=1.38-5.22) compared to patients without portal venous thrombosis adjusted for age, etiology of cirrhosis, ECOG performance status, tumor size and CTP score.

A parsimonious model (**Model C**) was subsequently constructed with stepwise backward selection, removing one variable at a time from the full Model B in order of least significance, until all retained variables had p<0.05 (p for removal 0.05).). **Table 19** shows the results of the multivariable Cox regression analysis of Model C, with baseline covariates of portal vein thrombosis, etiology of hepatic cirrhosis and tumor size on the effect of relative hazard of death, stratified by ethnicity. As in Model B, we observed a

higher hazard ratio of death for patients with portal vein thrombosis (HR= 2.47, 95% CI= 1.32-4.64, p=0.01) and larger than 5 cm tumors (5-7.99 cm, HR= 3.16, 95% CI=1.27-7.90, p=0.01; 8-11.99 cm, HR=3.37, 95% CI=1.37-8.30, p=0.01; >12 cm, HR=3.56, 95% CI= 2.46-22.53, p<0.0001).

The best fit of the three models was determined by the Akaike Information Criterion (AIC, (Akaike, 1974) and the Bayesian Information Criterion (BIC, (Schwarz, 1978). Models B and C have comparably low AIC and BIC values, with Model C having the lowest AIC and BIC values and being more parsimonious (**Table 20**).

Table 17. Multivariable Cox regression analysis of the hazard ratio for death, adjusted for baseline covariates of baseline age, ethnicity, etiology of cirrhosis, ECOG status, presence of portal venous thrombosis, tumor size and CTP score (Model A). HR=Hazard Ratio, LCI=lower 95% CI, UCI=Upper 95% CI, P-value=Wald test P-value.

MODEL A	HR	z	P-value	LCI	UCI
Age (years)					
16-37.5	1				
38-59	0.89	-0.10	0.92	0.10	7.63
60-80	1.22	0.18	0.86	0.15	9.87
>80	1.35	0.23	0.82	0.10	17.66
Ethnicity					
Caucasian	1.00				
African American	1.00	0.00	0.998	0.46	2.16
Asian	0.34	-1.67	0.10	0.09	1.21
Other	0.27	-1.79	0.07	0.06	1.14
Hepatic Cirrhosis					
HCV	1.00				
HBV	0.58	-1.24	0.22	0.25	1.37
Unknown/cryptogenic	0.42	-1.68	0.09	0.16	1.15
HBV and Alcohol	2.92	1.65	0.10	0.82	10.49
Other*	1.02	0.04	0.97	0.41	2.52
Baseline ECOG status					
0	1.00				
1 or 2	1.25	0.35	0.723	0.36	4.37
Baseline Portal Vein Thrombosis					
No	1.00				
Yes	2.77	3.03	**0.002**	1.43	5.37
Baseline Tumor Size (cm)					
2-4.99	1.00				
5-7.99	3.62	2.72	**0.007**	1.43	9.15
8-11.99	3.24	2.53	**0.011**	1.30	8.08
12-16	9.51	3.69	**0.000**	2.88	31.41

Baseline CTP score					
A	1.00				
B	1.31	0.45	0.65	0.40	4.29
C	0.49	-0.65	0.52	0.06	4.19

*Includes combination of alcoholic and HCV cirrhosis, combination of alcoholic and HBV/HCV cirrhosis, hemochromatosis, hemochromatosis and HBV, hemochromatosis and HCV, non-alcoholic steatohepatitis, or none.

Table 18. Comparison of unadjusted and adjusted hazard ratios of variables for confounding estimation according to the change-in-estimate method. HR=Hazard Ratio, LCI=lower 95% CI, UCI=Upper 95% CI, P-value=Wald test P-value, Change %: percent change of unadjusted minus adjusted HR for each variable that entered the multivariable time-independent Model A.

	HR	**P-value**	**LCI**	**UCI**	**HR**	**P-value**	**LCI**	**UCI**	**Change, %**
Univariate					**Multivariable**				
Age (years)									
16-37.5	1.00				1.00				
38-59	5.49	**0.09**	0.75	40.22	0.89	0.92	0.10	7.63	83.79
60-80	5.30	**0.10**	0.73	38.61	1.22	0.86	0.15	9.87	76.98
>80	4.83	**0.16**	0.54	43.36	1.35	0.82	0.10	17.66	72.05
Gender									
Male	1.00								
Female	1.01	0.98	0.57	1.80					
Ethnicity									
Caucasian	1.00				1.00				
African American	1.07	0.82	0.59	1.93	1.00	0.998	0.46	2.16	6.54
Asian	0.94	0.89	0.40	2.19	0.34	0.10	0.09	1.21	63.83
Other*	0.47	**0.15**	0.17	1.32	0.27	0.07	0.06	1.14	42.55
Hepatic Cirrhosis									

HCV	1.00				1.00				
Unknown/ cryptogenic	0.95	0.88	0.54	1.70	0.58	0.22	0.25	1.37	
HBV	0.59	**0.16**	0.28	1.22	0.42	0.09	0.16	1.15	
HBV and Alcohol	1.32	0.58	0.49	3.57	2.92	0.10	0.82	10.49	
Other*	0.56	**0.11**	0.27	1.13	1.02	0.97	0.41	2.52	
Baseline ECOG score									
0	1.00				1.00				
1	1.96	**0.004**	1.23	3.10	1.25	0.723	0.36	4.37	36.22
Baseline Metastases									
None	1.00								
Regional Lymph nodes	0.70	0.33	0.35	1.43					
Distant Metastasis	0.66	0.24	0.33	1.33					
Baseline PVT									
No	1.00				1.00				
Yes	2.07	**0.004**	1.25	3.430	2.77	**0.002**	1.43	5.37	-33.56
Baseline Tumor Burden									

Single Tumor	1.00								
2-3 tumors	0.91	0.81	0.44	1.91					
>3 tumors	0.91	0.68	0.57	1.45					
Baseline Tumor Size (cm)									
2-4.99	1.00				1.00				
5-7.99	2.70	**0.023**	1.14	6.37	3.62	**0.007**	1.43	9.15	-34.07
8-11.99	2.60	**0.016**	1.19	5.69	3.24	**0.011**	1.30	8.08	-24.62
12-16	4.85	**0.000**	2.05	11.49	9.51	**0.000**	2.88	31.41	-96.08
Baseline CTP score									
A	1.00				1.00				
B	2.07	**0.002**	1.30	3.28	1.31	0.65	0.40	4.29	36.71
C	0.75	0.77	0.10	5.45	0.49	0.52	0.06	4.19	34.67
1st mRECIST									
CR	1.00								
PR	0.71	0.32	0.36	1.40					
SD	1.07	0.80	0.62	1.86					
PD	0.58	0.29	0.22	1.57					
1st RECIST									
PR	1.00								

SD	1.35	0.27	0.79	2.30					
PD	0.76	0.59	0.28	2.06					

*Includes combination of alcoholic and HCV cirrhosis, combination of alcoholic and HBV/HCV cirrhosis, hemochromatosis, hemochromatosis and HBV, hemochromatosis and HCV, non-alcoholic steatohepatitis, or none.

Table 19. Multivariable Cox regression analysis of the hazard ratio for death adjusted for baseline covariates and stratified by ethnicity (**Model B**), so as to minimize PH non-proportionality of this variable (Schoenfeld global test for African-American ethnicity, p=0.04). Baseline portal venous thrombosis and baseline tumor size remain as statistically significant covariates of overall survival, with slight HR reductions of the risk of death. HR=Hazard Ratio, LCI=lower 95% CI, UCI=Upper 95% CI, P-value=Wald test P-value.

MODEL B	**HR**	**z**	**P-value**	**LCI**	**UCI**
Age (years)					
16-37.5	1				
38-59	1.135	0.120	0.908	0.133	9.679
60-80	1.418	0.320	0.745	0.172	11.689
>80	1.835	0.470	0.641	0.143	23.565
Baseline Portal Vein Thrombosis					
No	1.000				
Yes	2.683	2.910	**0.004**	1.379	5.218
Baseline CTP score					
A	1.000				
B	1.360	0.510	0.608	0.421	4.394
C	0.556	-0.530	0.595	0.064	4.851
Hepatic Cirrhosis					
HCV	1.000				
HBV	0.546	-1.170	0.241	0.198	1.503
Unknown/cryptogenic	0.672	-0.890	0.375	0.279	1.617
HBV and Alcohol	2.640	1.410	0.158	0.686	10.154
Other*	1.298	0.560	0.575	0.521	3.237
Baseline ECOG score					
0	1.000				
1o r 2	1.039	0.060	0.952	0.303	3.562
Baseline Tumor Size (cm)					
<4.99	1.000				
5-7.99	3.097	2.360	**0.018**	1.210	7.930
8-11.99	3.178	2.420	**0.015**	1.247	8.100
12-16	7.396	3.210	**0.001**	2.181	25.079
Stratified by Ethnicity					

*Includes combination of alcoholic and HCV cirrhosis, combination of alcoholic and HBV/HCV cirrhosis, hemochromatosis, hemochromatosis and HBV, hemochromatosis and HCV, non-alcoholic steatohepatitis, or none.

Table 20. Multivariable Cox regression analysis of the hazard ratio for death adjusted for baseline covariates of portal vein thrombosis, etiology of hepatic cirrhosis and tumor size **(Model C**), stratified by ethnicity (as in Model B, Table 19.). Stratification by ethnicity was performed so as to minimize non-proportionality for the African-American ethnicity variable (Schoenfeld test p-value=0.04). HR=Hazard Ratio, LCI=lower 95% CI, UCI=Upper 95% CI, P-value=Wald test P-value.

MODEL C	**HR**	**z**	**P-value**	**LCI.**	**UCI**
Portal Vein Thrombosis					
No	1	(base)			
Yes	2.47	2.82	**0.01**	1.32	4.64
Hepatic Cirrhosis					
HCV	1.00	(base)			
HBV	0.49	-1.48	0.14	0.19	1.26
Unknown/cryptogenic	0.67	-1.01	0.31	0.31	1.45
HBV and Alcohol	2.42	1.34	0.18	0.67	8.82
Other	1.38	0.73	0.46	0.59	3.24
Tumor Size (cm)					
2-4.99	1.00	(base)			
5-7.99	3.16	2.47	**0.01**	1.27	7.90
8-11.99	3.37	2.64	**0.01**	1.37	8.30
12-16	7.45	3.56	**0.00**	2.46	22.53
					Stratified by Ethnicity

*Includes combination of alcoholic and HCV cirrhosis, combination of alcoholic and HBV/HCV cirrhosis, hemochromatosis, hemochromatosis and HBV, hemochromatosis and HCV, non-alcoholic steatohepatitis, or none.

Table 21. Comparison of model fitting and adequacy for multivariable time-independent Cox Models A-C, according to Akaike information criterion (AIC) and Bayesian Information Criterion (BIC). Models with lowest AIC and BIC are preferable. Models B and C have similar AIC and BIC values. Obs=number of observations, ll(null)=log-likelihood of the null model, ll(model)=log-likelihood of the fitted model , df=degrees of freedom, AIC=Akaike Information Criterion, BIC= Bayesian Information Criterion.

Model	Obs	ll(null)	ll(model)	df	AIC	BIC
A	93	-204.87	-184.94	17	403.88	446.94
B	93	-152.26	-137.60	14	303.19	338.65
C	94	-156.39	-142.64	8	301.28	321.63

4.4.4. Time-dependent Univariate Cox Regression Analysis

Time-dependent covariates in the univariate Cox model included covariates that were collected longitudinally over time, during each visit. These included the variables of portal vein thrombosis, presence of metastases, tumor response according to mRECIST and RECIST, uni-dimensional longest diameter tumor size, and CTP score. In addition, the baseline variables of age, ethnicity, hepatic cirrhosis and ECOG performance status were also considered to be collected longitudinally, but did not change over time.

Time-dependent Cox regression analysis included a univariate Cox regression analysis to evaluate the association between each covariate (accounting for it varying over time) and the hazard for death. Univariate Cox regression analysis showed that the hazard of death was significantly higher for older patients, compared to patients younger than 37.5 years old (38-59 years old, HR=5.36, 95% CI=0.64-45.16, p=0.12; 60-80 years old, HR= 4.72, 95% CI=0.57-38.56, p=0.15), patients with ECOG score of 1 or 2, compared to 0 (HR=1.88, 95% CI=1.19-2.95, p=0.01) and patients with CTP score C, compared to patients with CTP score A (HR=2.06, 95% CI=0.89-4.80, p-0.09). Patients with ethnicity other than Caucasian, African American or Asian had lower risk of death compared to Caucasians (HR= 0.39, 95% CI=0.11-1.39, p=0.15). Patients with distant metastases had lower risk of death compared to those without (HR=0.49, 95% CI=0.27-0.89, p=0.02). Patients with enlarged regional lymph nodes had also lower risk of death compared to those without (HR=0.28, 95% CI=0.07-1.19, p=0.09). Patients with HBV-related cirrhosis had lower risk of death compared to patients with HCV-related cirrhosis

(HR= 0.57, 95% CI=0.27-1.19, p=0.13). Patients with progressive disease according to mRECIST had also lower risk of death compared to those with partial response (HR=0.53, 95% CI=0.25-1.12, p=0.1). **Table 21** shows the results of the univariate Cox regression analysis for each time-dependent covariate.

Table 22. Univariate Cox regression analysis of time-dependent covariates and hazard ratio for death during the study period of patients with unresectable HCC treated with TACE. Values are unadjusted. HR=Hazard Ratio LCI=lower 95% CI, UCI=Upper 95% CI, P-value=Wald test P-value.

	HR	z	P-value	LCI	UCI
Age (years)					
16-37.5	1	(base)			
38-59	5.36	1.54	**0.12**	0.64	45.16
60-80	4.72	1.44	**0.15**	0.57	38.96
>80	5.01	1.24	0.22	0.39	64.71
Gender					
Male	1	(base)			
Female	1.15	0.49	0.62	0.67	1.97
Ethnicity					
Caucasian	1	(base)			
African American	1.22	0.62	0.54	0.65	2.30
Asian	1.10	0.25	0.80	0.54	2.25
Other*	0.39	-1.46	**0.15**	0.11	1.39
Hepatic Cirrhosis					
HCV	1.00	(base)			
HBV	0.57	-1.50	**0.13**	0.27	1.19
Unknown/cryptogenic	0.93	-0.25	0.80	0.53	1.63
HBV and Alcohol	1.06	0.12	0.90	0.39	2.89
Other	0.48	-2.08	**0.04**	0.24	0.96
ECOG score					
0	1	(base)			
1 or 2	1.98	3.07	**0.002**	1.28	3.07
Portal Vein Thrombosis					
No	1	(base)			
Yes	1.22	0.85	0.39	0.77	1.93
Extrahepatic Metastases					
None	1	(base)			
Regional lymph nodes	0.69	-1.03	0.30	0.34	1.40
Distant Metastasis	0.72	-1.43	**0.15**	0.47	1.12q
mRECIST					

CR	1	(base)			
PR	0.95	-0.14	0.89	0.46	1.95
SD	0.74	-0.95	0.34	0.40	1.38
PD	0.53	-1.67	**0.10**	0.25	1.12
RECIST					
PR	1	(base)			
SD	0.84	0.56	0.47	1.50	0.56
PD	0.56	0.13	0.26	1.18	0.13
CTP score					
A	1	(base)			
B	1.09	0.35	0.73	0.67	1.78
C	2.06	1.68	**0.09**	0.89	4.80
Tumor Size (cm)					
2-4.99	1	(base)			
5-7.99	0.77	-0.86	0.39	0.42	1.40
8-11.99	1.02	0.06	0.95	0.55	1.88
12-16	0.94	-0.17	0.87	0.44	1.99

*Includes combination of alcoholic and HCV cirrhosis, combination of alcoholic and HBV/HCV cirrhosis, hemochromatosis, hemochromatosis and HBV, hemochromatosis and HCV, non-alcoholic steatohepatitis, or none.

4.4.5. Time-dependent Multivariable Cox Regression Analysis

Time-dependent multivariable Cox regression analysis was performed to evaluate how time-varying covariates jointly affect patient survival. For direct comparison of the multivariable time-independent estimates, two multivariable time-dependent models were examined; Model A and Model B. For Time-dependent **Model A**, statistically significant covariates ($p<0.20$) from the univariate time-dependent Cox regression analyses entered the multivariable time-dependent Cox regression analysis. These included the covariates of ethnicity, type of cirrhosis, ECOG status, extrahepatic metastases, mRECIST and CTP scores. Stratification by age was performed, to reduce PH non-proportionality (Schoenfeld global test for age, $p=0.0046$). For Time-dependent **Model B**, all covariates from the multivariable time-independent Cox regression analysis of baseline covariates (time-independent Model A) were entered.

Table 22 shows the results of the time-dependent multivariable Cox regression analysis of time-varying covariates (**Time-dependent Model A**) that achieved statistical significance ($p<0.2$) in the univariate analysis, on the relative hazard of death. **Table 23**. shows the results of the time-dependent multivariable Cox regression analysis of time-varying covariates (**Time-dependent Model B**) that entered the multivariable Cox regression analysis of baseline covariates (**Model A** of **Table 18.)**. **Table 24** shows the comparison between the time-independent and time-dependent multivariable Cox regression analyses. While baseline portal venous thrombosis and baseline tumor size were identified as statistically significant predictors of overall survival among time-

independent covariates, ECOG performance status and CTP score were identified as statistically significant predictors of overall survival among time-dependent predictors.

Table 23. Multivariable Cox regression analysis of the hazard ratio for death, adjusted for time-dependent covariates (**Model A)** of hepatic cirrhosis, ECOG status, portal venous thrombosis, extrahepatic metastases, tumor response according to mRECIST and CTP score. Baseline ECOG status and CTP score are statistically significant predictors of overall survival (p=0.02, respectively). HR=Hazard Ratio LCI=lower 95% CI, UCI=Upper 95% CI, P-value=Wald test P-value.

Time-dependent MODEL A					
	HR	**z**	**P-value**	**LCI**	**UCI**
Hepatic Cirrhosis					
HCV	0.90	-0.32	0.75	0.46	1.74
HBV	0.95	-0.15	0.88	0.48	1.86
Unknown/cryptogenic	1.51	0.85	0.40	0.58	3.89
HBV and Alcohol	0.54	-1.57	0.12	0.25	1.16
Other*	0.90	-0.32	0.75	0.46	1.74
ECOG score					
0	1.00	(base)			
1 or 2	1.83	2.36	**0.02**	1.11	3.03
Extrahepatic Metastases					
Absent	1.00	(base)			
Regional lymph nodes	0.93	-0.20	0.84	0.49	1.80
Distant Metastasis	0.69	-1.48	0.14	0.43	1.13
Portal Vein Thrombosis					
No	1.00	(base)			
Yes	1.26	1.43	0.15	0.92	1.74
mRECIST					
CR	1.00	(base)			
PR	1.16	0.69	0.49	0.77	1.74
SD	1.38	1.62	0.11	0.93	2.05
PD	0.76	-0.81	0.42	0.40	1.46
CTP score					
A	1.00	(base)			
B	1.46	2.33	**0.02**	1.06	2.01
C	1.24	0.56	0.57	0.59	2.58

*Includes combination of alcoholic and HCV cirrhosis, combination of alcoholic and HBV/HCV cirrhosis, hemochromatosis, hemochromatosis and HBV, hemochromatosis and HCV, non-alcoholic steatohepatitis, or none.

Table 24. Multivariable Cox regression analysis of the hazard ratio for death, adjusted for time-dependent covariates (Time-dependent **Model B**) of age, ethnicity, hepatic cirrhosis, ECOG status, portal venous thrombosis, extrahepatic metastases, tumor size and CTP score. ECOG score of 1 (p=0.05) and CTP score of B (p=0.02) are independent predictors of overall survival. HR=Hazard Ratio, LCI=lower 95% CI, UCI=Upper 95% CI, P-value=Wald test P-value.

Time-dependent MODEL B	**HR**	**z**	**P-value**	**LCI**	**UCI**
Age (years)					
16-37.5	1.00	(base)			
38-59	3.84	1.19	0.23	0.42	35.14
60-80	4.22	1.29	0.20	0.47	37.50
>80	5.71	1.39	0.16	0.49	65.99
Ethnicity					
Caucasian	1.00				
African American	1.03	0.08	0.94	0.50	2.12
Asian	1.10	0.22	0.83	0.47	2.57
Other	0.52	-1.17	0.24	0.18	1.55
Hepatic Cirrhosis					
HCV	1.00				
HBV	0.76	-0.75	0.45	0.37	1.56
Unknown/cryptogenic	0.80	-0.63	0.53	0.40	1.60
HBV and Alcohol	1.67	0.94	0.35	0.57	4.87
Other	0.56	-1.48	0.14	0.26	1.21
ECOG status					
0	1.00				
1 or 2	1.80	1.96	**0.05**	1.00	3.25
Portal Vein Thrombosis					
No	1.00				
Yes	1.04	0.19	0.85	0.71	1.51
Tumor Size					
2-4.99 cm	1.00				
5-7.99 cm	0.98	-0.06	0.95	0.62	1.58
8-11.99 cm	0.95	-0.23	0.82	0.60	1.49
12-16 cm	1.25	0.80	0.43	0.72	2.16

CTP score					
A	1.00				
B	1.50	2.32	**0.02**	1.07	2.12
C	1.67	1.48	0.14	0.85	3.27

*Includes combination of alcoholic and HCV cirrhosis, combination of alcoholic and HBV/HCV cirrhosis, hemochromatosis, hemochromatosis and HBV, hemochromatosis and HCV, non-alcoholic steatohepatitis, or none.

Table 25. Comparison of results between time-independent and time-dependent multivariable Cox regression analyses of the hazard ratio for death. Variables that were included in the multivariable Cox regression analysis of baseline covariates were considered for this comparison. HR=Hazard Ratio, LCI=lower 95% CI, UCI=Upper 95% CI, P-value=Wald test P-value.

	HR	**z**	**P-value**	**LCI**	**UCI**	**HR**	**z**	**P-value**	**LCI**	**UCI**
Age (years)	**Time independent**					**Time-dependent**				
16-37.5	1.00					1.00				
38-59	0.89	-0.1	0.92	0.1	7.63	3.84	1.19	0.23	0.42	35.14
60-80	1.22	0.18	0.86	0.15	9.87	4.22	1.29	0.20	0.47	37.50
>80	1.35	0.23	0.82	0.1	17.66	5.71	1.39	0.16	0.49	65.99
Ethnicity										
Caucasian	1.00					1.00				
African American	1.00	0	0.99	0.46	2.16	1.03	0.08	0.94	0.50	2.12
Asian	0.34	-1.67	0.1	0.09	1.21	1.10	0.22	0.83	0.47	2.57
Other*	0.27	-1.79	0.07	0.06	1.14	0.52	-1.17	0.24	0.18	1.55
Etiology of Cirrhosis										
HCV	1.00					1.00				
HBV	0.58	-1.24	0.22	0.25	1.37	0.76	-0.75	0.45	0.37	1.56
Unknown /cryptogenic	0.42	-1.68	0.09	0.16	1.15	0.80	-0.63	0.53	0.40	1.60
HBV and Alcohol	2.92	1.65	0.1	0.82	10.49	1.67	0.94	0.35	0.57	4.87
Other	1.02	0.04	0.97	0.41	2.52	0.56	-1.48	0.14	0.26	1.21
ECOG status										
0	1.00					1.00				
1	1.25	0.35	0.72	0.36	4.37	1.80	1.96	**0.05**	1.00	3.25
PVT										
No	1.00					1.00				
Yes	2.77	3.03	**0.002**	1.43	5.37	1.04	0.19	0.85	0.71	1.51

Tumor Size (cm)										
2-4.99	1.00					1.00				
5-7.99	3.62	2.72	**0.007**	1.43	9.15	0.98	-0.06	0.95	0.62	1.58
8-11.99	3.24	2.53	**0.01**	1.3	8.08	0.95	-0.23	0.82	0.60	1.49
12-16	9.51	3.69	**0.00**	2.88	31.41	1.25	0.80	0.43	0.72	2.16
CTP score										
A	1.00					1.00				
B	1.31	0.45	0.65	0.4	4.29	1.50	2.32	**0.02**	1.07	2.12
C	0.49	-0.65	0.52	0.06	4.19	1.67	1.48	0.14	0.85	3.27

*Includes combination of alcoholic and HCV cirrhosis, combination of alcoholic and HBV/HCV cirrhosis, hemochromatosis, hemochromatosis and HBV, hemochromatosis and HCV, non-alcoholic steatohepatitis, or none.

CHAPTER 5. DISCUSSION AND CONCLUSIONS

5.1. Clinical implications

The purpose of this work was to determine whether baseline and longitudinally collected clinical and imaging data, tumor response to therapy and liver function are predictive of overall survival in patients with unresectable HCC treated with TACE. We first assessed whether time-independent (baseline) covariates are predictive of overall survival and identified that baseline portal vein thrombosis and baseline uni-dimensional tumor size are predictive of overall survival. We subsequently assessed whether time-dependent (longitudinal) covariates are predictive of overall survival and identified that ECOG performance status and CTP score as statistically significant predictors of overall survival. Since the time-dependent multivariable Cox regression models have almost 3 times more observations (in the form of repeated measures) than the time-independent multivariable Cox regression models, the former may provide with more robust information associated with the temporal changes in information of selected variables. The time-dependent multivariable Cox regression models illustrated in this work may provide a useful example and guidance for clinicians wishing to further explore the value of longitudinally collected covariates and their effect on overall survival.

5.1.1. Predictors of overall survival according to time-dependent Cox PH models

5.1.1.1. ECOG performance status

Our findings from the time-dependent analysis of the longitudinal study suggest a higher hazard of death for patients with ECOG status of 1, as compared to an ECOG status of 0 (time-dependent multivariable Model A, HR=1.83, 95% CI=1.11-3.03, p=0.02; time-dependent multivariable Model B, HR=1.80, 95% CI=1.00-3.25, p=0.05). This is in accordance with two very recently published research studies as well as the 2015 Japanese clinical guidelines, suggesting that TACE should be reserved for patients with ECOG performance status of 0 (Hsu et al., 2013; Nishikawa et al., 2015; R. T. Poon et al., 2015). None of the other clinical studies, however, utilized time-dependent Cox PH models for assessing the effect of ECOG status over time on overall survival.

5.1.1.2. Child-Turcotte Pugh score

Our findings from the time-dependent analysis of the longitudinal study suggest a higher hazard of death for patients with CTP score of B, as compared to CTP score of A (time-dependent multivariable Model B, HR=1.50, 95% CI=1.07-2.12, p=0.02). Patients with CTP score of C had an even higher hazard of death (HR=1.67, 95% CI=0.85-3.27, p=0.14), but results did not reach statistically significance due to the small sample size of patients with CTP score of C. On the numerical CTP score scale, we observed statistically significant changes in the CTP score over time, particularly at 6 months and at study exit. This is in accordance with previously published research on the prognosis of the co-existence of two disease entities in patients with HCC, namely cancer and

cirrhosis, on overall survival(Barman et al., 2014; Brown et al., 2004). Similarly, none of the clinical studies, however, utilized time-dependent Cox PH models for assessing the effect of CTP score over time on overall survival.

5.1.2. Predictors of overall survival according to time-independent baseline covariate data analysis

5.1.2.1. Portal vein thrombosis

Our findings from the multivariable Cox regression analysis of time-independent (baseline) covariates suggest that baseline portal vein thrombosis is an independent predictor of overall survival in patients with unresectable HCC treated with TACE ((HR= 2.47, 95% CI= 1.32-4.64, p=0.01), after adjusting for hepatic cirrhosis (time-independent parsimonious Model C). Patients with portal vein thrombosis had a median survival of 10 months, compared to a median survival of 23 months in patients without portal vein thrombosis (p=0.003, log rank test). This finding is in accordance with previous studies, suggesting that patients with portal vein thrombosis have worse overall survival, compared to patients without portal vein thrombosis(Georgiades, Hong, D'Angelo, & Geschwind, 2005).

5.1.2.2. Longest uni-dimensional tumor size

Our study indicates that the longest uni-dimensional baseline tumor size is an independent predictor of overall survival. Compared to patients with tumor longest

diameter of less than 5 cm, patients with tumors 5-7.99 cm had a higher hazard of death (HR= 3.16, 95% CI=1.27-7.90, p=0.01), after adjusting for etiology of cirrhosis. Accordingly, patients with baseline tumor sizes larger than 8 cm and smaller than 12 cm had a similarly higher hazard of death compared to patients with baseline tumor sizes smaller than 5 cm (HR=3.37, 95% CI=1.37-8.30, p=0.01), after adjusting for etiology of cirrhosis. Patients with tumors larger than 12 cm had a much higher hazard of death compared to patients with baseline tumor sizes smaller than 5 cm (HR=7.45, 95% CI= 2.46-22.53, p<0.0001), after adjusting for etiology of cirrhosis. This finding suggests that tumors with longest diameters between 5 and 12 cm may show a similar biological behavior. After regrouping patients according to the cut-off value of 5 cm in uni-dimensional tumor size, patients with smaller tumors had a statistically significant higher hazard of death (adjusted HR=3.60, 95% CI=1.60-8.09, p=0.002). The prognostic impact of uni-dimensional tumor size measurements on overall survival has been demonstrated in rather few studies, as most research studies focus on documenting tumor response by the established RECIST and mRECIST. While uni-dimensional measurements are thought to be a poor surrogate for tumor volume and tumor size measurements are challenging to reliably reproduce, we demonstrated that the cut-off value of 5 cm is an independent prognostic factor of overall survival. This cut-off value, however, already part of the Milan criteria for liver transplantation, represents the distinct biology of small HCC tumors(Yao et al., 2002).

5.2. Statistical implications

5.2.1. Comparison of time-dependent and time independent multivariable Cox regression analyses

Cox proportional hazards regression analyses are very common in medical research and particularly, in oncology. However, these analyses do not usually utilize all of the available information and are often restricted to data collected at baseline. In clinical oncologic practice, individual clinical, biochemical and imaging data are routinely collected for each patient at frequent time points after study entry, however, this added information is rarely examined in relation to survival. Yet, a key clinical question is that of prognosis, especially in regards to the variation in prognostic values over time. Time-dependent multivariable Cox regression is a robust option for assessing the temporal dynamics of covariate effects on right-censored failure times.

The use of time-dependent Cox models in this work resulted in identification of different predictors of overall survival than those identified in time-independent models, probably due to the fact that the latter do not account for changes in tumor response, clinical performance status and liver function over time. If only baseline measurements are available, then ECOG score of zero, absence of baseline PVT and smaller baseline tumor size are significantly associated with longer overall survival, after adjusting for other potential confounders. In the time-dependent analysis where covariate values may vary over time, lower ECOG score and CTP score of A are significantly associated with

longer overall survival; PVT and tumor size are not longer significantly associated with survival. This work suggests the importance of collecting both ECOG score and CTP score, if possible, over the course of treatment in predicting future prognosis. .

5.3. Study limitations

One major study limitation is the retrospective nature of the analysis. Obtaining the clinical data via chart review is limited by access and accuracy of the data. Additionally, as a retrospective study, it is subject to selection bias and incomplete data collection.

For this study, we performed complete case analysis and excluded patients with missing values of tested covariates. This resulted in exclusion of useful data that had been recorded. When data can be assumed as missing at random, complete case analysis, however, is thought to provide unbiased estimates of the parameters of interest, although with reduced precision. Alternative methods of dealing with missing data need to be further explored, so as to identify patterns of missingness as well as missing data mechanisms.

5.4. Study Strengths

A major strength of this study is the assessment of longitudinally collected patient data. Important events that may occur during follow-up, such as a change in performance status or in liver function should not be ignored, as they may lead to biased results. Assessment of longitudinally collected data in patients with HCC treated with TACE has

not been extensively reported and this study may aid as a guide for clinicians who wish to further explore the wealth and value of such routinely available data.

Studies with applications of time-dependent Cox models are still few, but have increased over the past years. However, several of these, have dealt only with the special case of a single time-varying variable. In this study, we simultaneously explored several time-varying variables in time-dependent Cox models that can handle well both categorical and continuous variables. The simultaneous exploration of time-varying covariates related both to the tumor and liver damage is essential in this category of cancer patients, where hepatocellular cancer co-exists with hepatic cirrhosis.

5.5. Conclusions

In this retrospective study of 211 patients with unresectable HCC treated with TACE, we examined, in a subset of 119 patients with complete case analysis, the effect of baseline and longitudinal time-dependent predictors on overall survival. Mean overall survival was 18.85 months and the median was 14 months. The analysis of time-independent Cox models revealed that portal vein thrombosis (HR= 2.47, 95% CI= 1.32-4.64, p=0.01) and uni-dimensional tumor size greater than 5 cm (5-7.99 cm, HR= 3.16, 95% CI=1.27-7.90, p=0.01; 8-11.99 cm, HR=3.37, 95% CI=1.37-8.30, p=0.01; >12 cm, HR=3.56, 95% CI= 2.46-22.53, p<0.0001) were independent baseline predictors of overall survival, after adjusting for hepatic cirrhosis. The analysis of time-dependent Cox models revealed a higher hazard of death for patients with ECOG status of 1 or 2, as compared to an ECOG status of 0 (time-dependent multivariable Model A, HR=1.83,

95% CI=1.11-3.03, p=0.02, adjusted for hepatic cirrhosis, metastases, portal vein thrombosis, mRECIST and CTP score, stratified by age); time-dependent multivariable Model B, HR=1.80, 95% CI=1.00-3.25, p=0.05, adjusted for age, ethnicity, hepatic cirrhosis, portal vein thrombosis, tumor size and CTP score) and a higher hazard of death for patients with CTP score of B, as compared to CTP score of A (time-dependent multivariable Model B, adjusted HR=1.50, 95% CI=1.07-2.12, p=0.02, after adjusting for age, ethnicity, hepatic cirrhosis, portal vein thrombosis, tumor size and CTP score). By taking into account more information clustered per patient over time, time-dependent Cox proportional hazard models provide more accurate hazard ratio estimates of death than those obtained with time-independent analysis. The study of patients with unresectable HCC and cirrhosis treated with TACE using longitudinally collected clinical and imaging data in time-dependent Cox models may provide more reliable information about their prognosis.

CHAPTER 6. FUTURE WORK

6.1. Multiple Imputation for Missing Data

In this study, as noted in the limitations, we included only cases with complete covariate information over time in order to set up and assess the time-dependent Cox models. We, therefore, excluded 43.6% of patients from the original dataset, resulting in a substantical loss of information. Multiple imputation methods provide a useful strategy for dealing with data sets with missing values. Instead of filling in a single value for each missing value, Rubin's multiple imputation procedure (Rubin, 1976) replaces each missing value with a set of plausible values that represent the uncertainty regarding the correct imputed value. These multiply imputed data sets are then analyzed by using standard procedures for complete data and combining the results from these analyses. This results in valid statistical inferences that properly reflect the uncertainty due to missing values (Yuan, 2010).

6.2. Joint Modeling Approaches

Cancer studies often collect time-to-event data and repeated measurements of longitudinal data for each subject. Longitudinal data can be important predictors or surrogates of a time to event, such as overall survival. However, classical models such as

the linear mixed model for longitudinal data and the Cox proportional hazards model for time-to-event data do not consider the dependencies between these two different data types (longitudinal and time-to-event data). A powerful method that takes into account the dependency and association between longitudinal data and time-to-event data is joint models for longitudinal and time-to-event data. Joint models for longitudinal and time-to-event data are models that bring these two data types together (simultaneously) into a single model to better assess the effect of a treatment(Crowther, Abrams, & Lambert, 2013; Ibrahim, Chu, & Chen, 2010; Rizopoulos, 2013; D. Zhang et al., 2014). As a result of the rapid development of clinical and genetic biomarkers in clinical trials, joint modeling has gained in popularity in recent years because it reduces bias in estimates of the treatment effects and provides improvements of efficiency in the assessment of treatment effects and other prognostic factors.

List of Abbreviations

AASLD: American Association For The Study Of Liver Diseases

BCLC: Barcelona Clinic Liver Cancer

CI: Confidence Interval

CR: Complete Response

CT: Computed Tomography

CTP: Child-Turcotte-Pugh

DEB: Doxorubicin-Eluting Beads

EDA: Exploratory Data Analysis

Gd: Gadolinium

GDA: Gastroduodenal Artery

GRE: Gradient Recall Echo

HAE: Hepatic Arterial Embolization

HCC: Hepatocellular Carcinoma

HCV: Hepatitis C Virus

HIPAA: Health Insurance Portability and Accountability Act

HR: Hazard Ratio

INR: International Normalized Ratio

IRB: Institutional Review Board

JHH: Johns Hopkins Hospital

LOWESS: locally weighted scatterplot smoothing

MRI: Magnetic Resonance Imaging

OR: Odds Ratio

P-H: Proportional Hazards

PD: Progressive Disease

PR: Partial Response

RCT: Randomized Controlled Trial

RECIST: Response Evaluation Criteria In Solid Tumors

SD: Stable Disease

SE: Standard Error

SMA: Superior Mesenteric Artery

TACE: Transcatheter Arterial Chemoembolization

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Curriculum Vitae

Eleni Apostolos Liapi was born on July 28 1972, in Athens, Greece to Apostolos and Aikaterini Liapi. She graduated as valedictorian from the 2nd Lyceum, in Volos, Greece in 1990. She received her M.D. (Medical Degree, Ptychion Iatrikis) from the University of Ioannina Faculty of Medicine, Ioannina, Greece in 1996. She completed a year of medical residency in Internal Medicine/Pulmonology at the General Health System of Greece/Sotiria General Hospital of Thoracic Diseases in Athens, Greece in 1997, a residency in Diagnostic Radiology at the General Health System of Greece/University of Athens School of Medicine in 2003, and post-doctoral research fellowship in Interventional Radiology at the Johns Hopkins University School of Medicine Department of Radiology, Division of Interventional Radiology, in Baltimore, Maryland in 2008. In 2009, she was appointed as an NIH T32 trainee, at the Training Program for Physician Scientists in Imaging Research (PI: D. Wong), at the Department of Radiology at the Johns Hopkins University School of Medicine, in Baltimore, Maryland, and in 2010, she was appointed as an Instructor in Radiology at the Department of Radiology at the Johns Hopkins University School of Medicine, in Baltimore, Maryland.

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